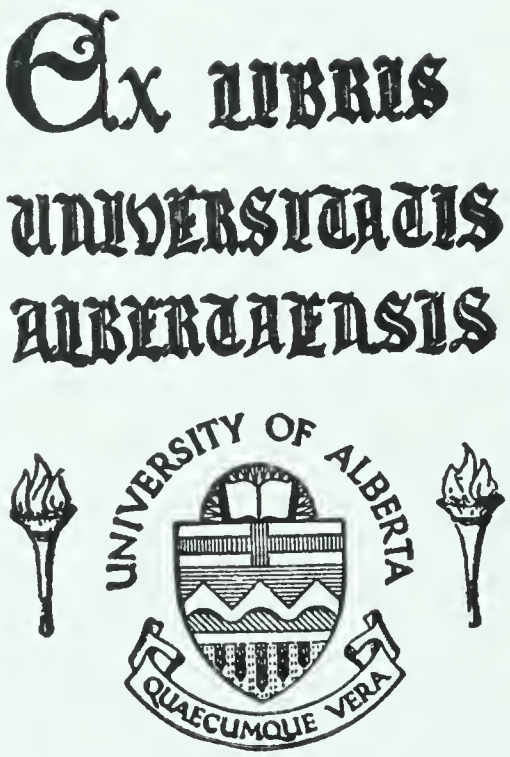


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THE UNIVERSITY OF ALBERTA

# BLOODFLOW TO THE WALKER 256 CARCINOMA

by

Alan Gerald Smith

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Bloodflow to the Walker 256 Carcinoma", submitted by Alan Gerald Smith in partial fulfilment of the requirements for the degree of Master of Science.

# ABSTRACT

The introduction of clinical techniques of regional intra-arterial chemotherapy has renewed interest in the magnitude and regulation of the blood circulation within malignant tumors. This problem has been investigated in the past with a number of indirect methods which have indicated that the bloodflow to tumors is greater than that to most normal tissues, and that tumor blood vessels respond differently to vasoactive chemicals than do those of normal tissue. In the following study, the vascular architecture, and the magnitude and regulation of capillary-bed bloodflow were investigated in a rapidly growing transplantable tumor, the Walker 256 carcinoma, implanted in the hind limb musculature or in the left kidney of Sprague-Dawley rats.

The blood vessels of this tumor were found to be relatively sparse, of small caliber, and peripherally distributed in femoral arteriograms and vinyl vascular casts of tumor-bearing limbs. Radioautographs of cross-sections of tumor-bearing limbs following the intra-arterial infusion of radiating ($Sc_{46}$) microspheres of 25 $\pm$ 5 microns in diameter revealed that no vessels of larger than 25 $\pm$ 5 microns diameter penetrated the tumor mass.

The capillary-bed bloodflow to the tumor, skeletal muscle and skin in the rat hind limb was estimated by indicator dilution techniques employing a diffusible indicator, Radio-4-Iodoantipyrine $I_{131}$ (Abbott). The bloodflow to the tumor (0.27 $\pm$ 0.009 ml/min/gm.)

was found to be less than that to resting skeletal muscle (0.36 ± 0.008), and only slightly greater than that to skin (0.23 ± 0.008). The sources of error involved in this method were studied and found to be of small magnitude.

Comparisons of the capillary bed flow to the intramuscular tumor (0.27 ± 0.009 ml/min/gm.) with those to the tumor as a renal implant (0.34 ± 0.017), and to lymphnode metastases from the hind limb tumor (0.29 ± 0.011) demonstrated that the tumor bloodflow is not influenced by the site of implantation. The normal renal bloodflow (1.86 ± 0.048) was found to be approximately six times greater than that to the renal tumor.

Changes in the tissue bloodflow to the tumor, skeletal muscle and to skin in the rat hind limb were studied following lumbar sympathectomy and following the systemic administration of epinephrine, l-norepinephrine, tolazoline, phenoxybenzamine, and chlorpromazine. Tumor, muscle and skin flows were increased by all stimuli except l-norepinephrine and phenoxybenzamine. Epinephrine produced an initial decrease and later marked increase in tissue flows to the tumor and to its host tissue (skeletal muscle) while producing a marked decrease in skin flows. The vessels of the tumor were found to be functionally identical to those of the host tissue in the intramuscular implant.

This tumor is therefore relatively inaccessible to materials delivered in the arterial bloodflow, a finding which may explain both the disappointing clinical results of regional antitumor chemotherapy and the occurrence of central necrosis in fast-growing tumors.

ACKNOWLEDGEMENTS

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2. This study was completed while a Research Fellow, Alberta Division, Canadian Cancer Society.

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# INTRODUCTION

The introduction and development of methods for the treatment of malignant tumors by the administration of chemotherapeutic agents into the vascular supply to the tumor over the past fourteen years has prompted considerable interest in the magnitude and mechanics of tumor bloodflow. Klopp and co-workers (45) introduced this topic when they noted a marked and localized reaction produced when nitrogen mustard was administered into the regional arterial supply, a reaction not evoked when even lethal doses were given by other routes. The frequent systemic toxic complications produced using this method indicated that much of the agent passed through the local capillary bed to reach the general circulation.

Creech, Ryan, and Krementz (13) in 1950, developed isolation-perfusion techniques to decrease this "leak" of the chemotherapeutic agent into the general circulation. The results of isolation-perfusion techniques, while dramatic in a few patients, have been discouraging. This relative failure of these techniques in clinical practice has generated considerable interest in a more thorough investigation into the mechanics of tumor bloodflow.

It has long been believed that neoplastic tissues

have a greater than normal bloodflow, a belief based upon the finding of large vascular plexuses around the periphery of tumors at surgery, on the demonstration of numerous vessels in histologic sections of tumor tissue, and on the reasonable supposition that rapidly growing tumor tissue must require large amounts of nutrients to support its presumably high metabolic rate.

The presence of numerous vascular channels in a tissue cannot be accepted as direct evidence of increased local blood supply to this tissue and may represent local arteriovenous shunts, may be a consequence of local venous obstruction, or some other factor which is equally unrelated to tissue (capillary bed) bloodflow. A distinction must therefore be made between vascularity and bloodflow, a distinction which has not been made in much of the earlier studies of tumor bloodflow.

There have been numerous investigations of tumor bloodflow, largely restricted to morphologic features of tumor vasculature (5, 8, 9, 14, 62, 73). These investigations utilizing a variety of methods of vascular casting or arteriography have demonstrated that many transplantable animal tumors and human pulmonary and hepatic metastases have an abnormal vasculature where the arterial supply is provided by small peripherally located vessels.

Conflicting evidence regarding the magnitude of tumor bloodflow has been provided by investigators utilizing a variety of indirect methods for the estimation of tumor bloodflow. Indirect evaluation of tumor bloodflow has been attempted using the distribution of Lyssamine green dye (21, 51), by observations of the effects of reducing arterial pressure on tumor growth (1), by the determination of oxygen tension in tumor tissue (69, 70), by comparing the arterio-venous oxygen differentials in regions bearing and not bearing tumors (7), and by the measurement of the skin temperature overlying superficial tumors (6, 50). None of these methods provide any measurement of the actual amount of blood flowing through the capillary bed of the tumor.

Gullino and Grantham (27), using indicator dilution methods employing $K_{42}Cl$ and by direct measurement of venous outflow from specially prepared tumors supplied with a single artery and vein (26), have shown that the blood supply to a number of transplantable animal tumors is considerably less than the bloodflow to most normal tissues. Similar results were reported by Rogers, Reagan, and Aust (53), using indicator dilution techniques employing Iodoantipyrine $I_{131}$. These were the first reports of the magnitude of tumor bloodflow based upon experimental techniques which

provide a direct measurement of tissue bloodflow.

This relatively low value of tumor bloodflow suggests that the tumor is comparatively inaccessible to chemotherapeutic agents delivered in the arterial blood supply, as the host organ receives a greater dose of the agent than the tumor. It therefore became of interest to investigate the response of tumor bloodflow to a variety of vasoactive chemicals in the hope of producing selective enhancement of tumor bloodflow, without a corresponding increase in the bloodflow to the host organ, and hence increasing the tumor exposure to chemotherapeutic agents delivered in the arterial blood.

Nataze and co-workers (50) studied the response of subcutaneous tumors to epinephrine, acetylcholine, and histamine by measuring changes in the skin temperature overlying the tumor, and by direct visualization of tumor and host organ vessels using transparent chambers. They reported that epinephrine produced an increase in tumor vessel diameters and in linear flow rates, and concluded that this drug produces an increase in tumor bloodflow. These workers found that acetylcholine and histamine had opposite effects. Similar conclusions were reported by Bierman et al (6). It is important to note that these are the opposite effects produced by these drugs in most normal tissues (2, 4, 15, 17).

Although the experimental method used by these workers is open to doubt, their findings strongly suggest that the vessels of the tumor are different from those of the host organ. Gullino and Grantham (28), however, using more precise techniques, were unable to demonstrate any difference in the response of the bloodflow to host organ or tumor to these and to other drugs.

The object of the following study is to investigate certain features of the vessels and bloodflow to a transplantable rat tumor, the Walker 256 carcinoma. The vascular architecture of the tumor will be investigated by arteriography, vascula r casts, and by radioautographic techniques, studying the caliber, arrangement and location of supply vessels. The magnitude and the regulation of tumor blood flow will be studied with indicator dilution techniques utilizing a diffusible indicator, 4-Iodoantipyrine ($I_{131}$), to determine the actual level of tumor bloodflow and to determine the response of tumor and host tissue bloodflows to a variety of vasoactive drugs.

# CHAPTER I

## METHODS FOR THE ESTIMATION OF TUMOR BLOODFLOW

It is of considerable clinical significance from the standpoint of antitumor chemotherapy by either systemic or regional routes to compare the magnitude of tumor bloodflow with that to other normal tissues. Should tumor bloodflow be very small compared to normal tissues, a relatively small amount of administered chemotherapeutic agent will be delivered to the tumor. This is especially important in therapy with drugs such as alkylating agents (4, 20), which become rapidly bound to tissue cells, and which have no special affinity for tumor tissue.

The experimental study of regional bloodflow and of regional vascular beds has provided a large number of methods for the estimation of regional bloodflow. In the following study, a number of these methods have been adapted for the estimation of the tissue bloodflow in an albino rat bearing a transplantable animal tumor, the Walker 256 carcinoma, implanted in the right hind limb musculature. The bloodflows to normal muscle and skin in the tumor bearing limb have been estimated and compared with that to the tumor.

These methods for estimating tissue bloodflows will be discussed under the following headings:

A. Arteriography of the tumor-bearing limb.

B. Injection-corrosion techniques.

C. Radio-autography of tumor vasculature.

D. Indicator-dilution techniques for measuring blood-flow.

The information regarding tissue bloodflow provided by these methods and the degree of accuracy in the result will be discussed for each method.

The transplantable tumor used in this study is a Walker 256 carcinoma which has been maintained in this laboratory by serial transplantation in rats of the Sprague-Dawley strain. The preparation of tumor utilized in the following investigations is outlined below:

Under diethyl ether anesthesia, approximately one gram of tumor tissue is removed from stock Sprague-Dawley rats bearing a six to eight day old Walker 256 carcinoma implanted in the right gastrocnemius muscle. This tumor tissue is finely minced in sterile physiological saline at room temperature. A tumor cell suspension is prepared by adding these tumor fragments to ten milliliters of physiological saline in a tissue homogenizer.

This resultant tumor cell suspension is utilized immediately. Transplantation is achieved by the local injection of 0.15 ml. of the tumor cell suspension into the chosen site in the experimental groups of rats. This dose

contains approximately two million tumor cells and ensures virtually one hundred percent tumor "takes".

For purposes of describing tumor age, the day of transplantation is taken as day zero.

The Walker 256 Carcinoma was chosen for this study as it is a rapidly growing tumor which can be transplanted to most tissues of the Sprague-Dawley rat (64), and which was readily available in our laboratory.

A. Arteriography of the Tumor Bearing Limb:

The intra-arterial administration of a radio-opaque substance and subsequent radiography provides a simple, although gross indication of the source and extent of the arterial blood supply to that region of the body. Bierman and co-workers (5) demonstrated that human hepatic metastases have a blood supply derived from small peripherally located vessels, using arteriographic methods.

These methods were applied to the study of the blood supply to the Walker 256 carcinoma implanted in the hindlimb musculature of a rat, to demonstrate these features of tumor blood supply. Arteriograms were prepared of normal and tumor bearing limbs for comparison.

Method:

Sprague-Dawley rats, bearing a six day old Walker

tumor implanted in the right gastrocnemius muscle were anesthetized with intra-peritoneal Pentobarbital sodium (30 mg/kg), and a PE50 polyethylene catheter was inserted into the right external iliac artery through a groin incision.

One group of rats received a rapid infusion of Hypaque sodium (0.5 ml. of 50%) through the cannula and simultaneous radiographs (Figure 1) were taken. All radiographs were taken on standard Kodak Xray film with an exposure of 42 KVA at 30 inches for one twentieth second.

A second group of rats prepared in the identical manner were infused with varying doses of metallic mercury (Figures 2, 3, 4, 5) and radiographs were made at the same exposure. Metallic mercury was used to provide better small vessel definition.

Results:

The femoral arteriogram of a tumor bearing leg infused with Hypaque sodium is shown in figure 1. The small vessel contrast provided by this agent is poor, with the presence of the dye in the vascular bed showing as a diffuse blush. The area bearing the tumor (arrow) contains less of the contrast material than the surrounding muscle and therefore presumably has less bloodflow.

The radiographs using metallic mercury (figures 2, 3,

Figure 1: Radiograph of the tumor-bearing limb following the infusion of 0.50 ml. of 50% Hypaque sodium into the right common iliac artery.

(arrow marks tumor location)

Figure 2: Radiograph of the tumor-bearing limb following the infusion of 0.20 ml. of metallic mercury into the right common iliac artery.

(arrow marks tumor location)

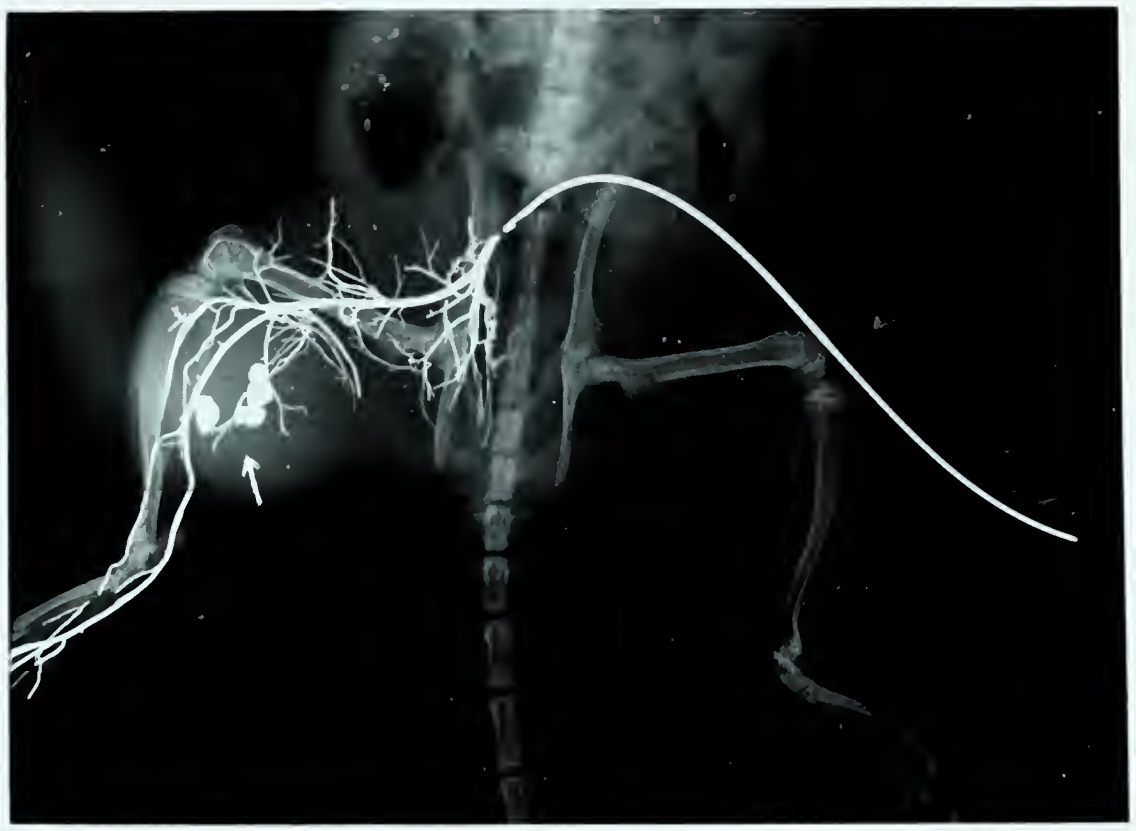

Figure 3: Radiograph of the tumor-bearing limb following the infusion of 0.40 ml. of metallic mercury into the right common iliac artery.

(arrow marks tumor location)

4, 5) show the distribution of the small vessels of the limb. The presence of the tumor (arrow) is indicated by a defect in the vascular pattern of the limb, particularly noticeable in figure four.

Discussion:

A comparison of the vascular architecture of normal and tumor-bearing legs was provided by the infusion of 5.0 ml. of metallic mercury into the abdominal sorta of an anesthetized rat bearing the standard tumor, as shown in figure 6.

Comparison of the two legs reveals that the number and routing of the major vessels in the leg is not altered by the presence of a six day old Walker 256 carcinoma, except that the vessels show some effects of stretching over the surface of the tumor.

The relative radio-lucency of the area bearing the tumor when compared with the surrounding muscle (Figure 1) offers support for the hypothesis that the tumor bloodflow is less than that to the surrounding muscle.

The "hole" in the small vessel pattern in the area occupied by the tumor (Figure 4, 5), when compared with the pattern of the normal leg (Figure 6), strongly suggests that the tumor is supplied by vessels of small size which enter the tumor from the periphery.

Figure 4: Radiograph of the tumor-bearing limb following the infusion of 0.60 ml. of metallic mercury into the right common iliac artery.

(arrow marks tumor location)

Figure 5: Radiograph of the tumor-bearing limb following the infusion of 1.0 ml. of metallic mercury into the right common iliac artery.

(arrow marks tumor location)

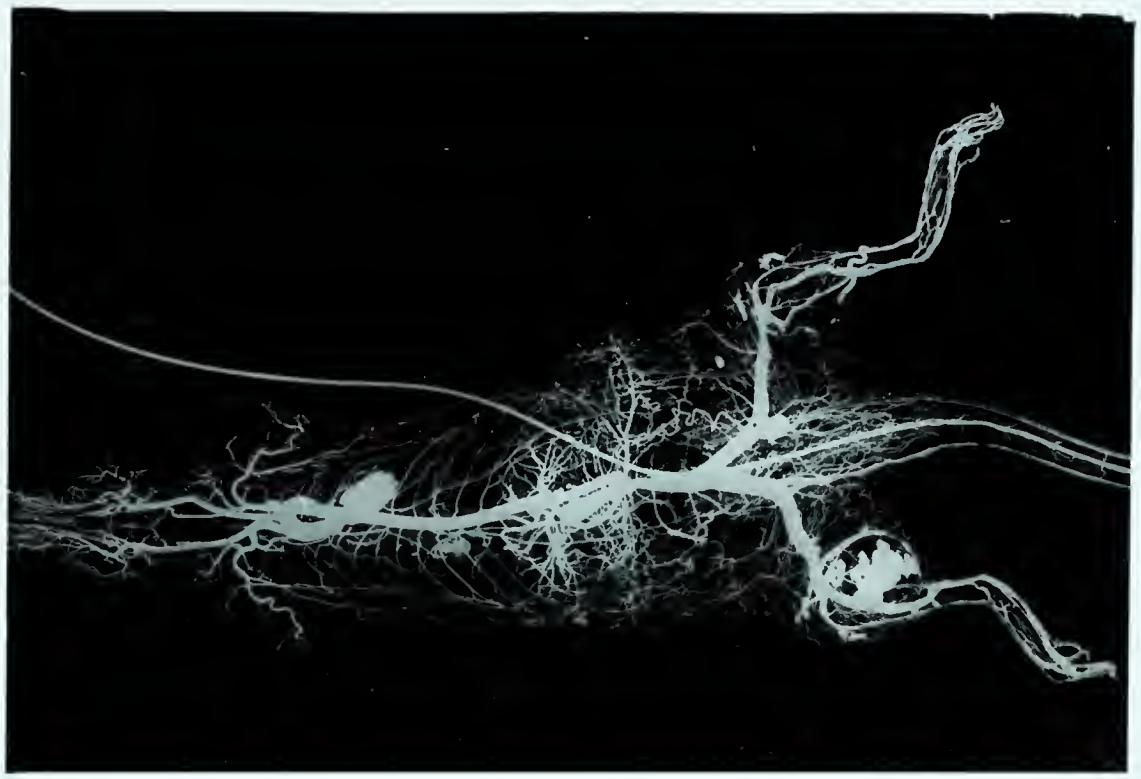

Figure 6: Radiograph of the hind limbs following the infusion of 5.0 ml. of metallic mercury into the abdominal aorta.

(arrow marks tumor location)

This finding of tumor blood supply through small peripherally located vessels has been noted in tumors in other sites, namely, in the liver (5, 8, 9), and in the lung (14).

Conclusions:

The Walker 256 carcinoma implanted in the hind limb musculature of the rat is not supplied with large arterial branches penetrating the tumor, but rather by small vessels entering the tumor from the periphery. The magnitude of the local blood flow to the tumor is probably less than that to the surrounding tissues of the leg.

B. Injection-Corrosion techniques.

The vascular architecture of the tumor in any site may be studied by the preparation of injection-corrosion casts of the arterial and venous circulations. The conclusions drawn from arteriography of tumor bearing legs in the preceding method; that the tumor receives its blood supply from small peripherally located vessels, may be confirmed by the examination of vascular casts of normal and tumor bearing limbs.

Method:

Vascular casts of normal and tumor bearing limbs were prepared in the following manner, utilizing a vinyl acetate

injection medium* (72).

Sprague-Dawley rats bearing a six day old Walker 256 carcinoma implanted in the right gastrocnemius muscle were used in this study. The tumor bearing rats were sacrificed with diethyl ether and PE50 polyethylene catheters were inserted into the abdominal aorta and the vena cava at the level of the renal vessels. Both hind limbs were cleared of blood by infusing saline followed by acetone into the aortic catheter. The arterial circulation was then filled with approximately 2.0 ml. of red colored vinyl acetate in acetone*.

The entire body of the rat was then immersed in warm tap water for three hours to harden the vinyl cast and both hind limbs were dissected free and corroded in concentrated potassium hydroxide for 24-48 hours. The resultant casts were defatted with ethyl alcohol and photographed (Figures 7 and 8).

Results and Discussion:

The vascular cast of the tumor bearing leg (Figure 7) reveals a deficiency or hole in the pattern in the area occupied by the tumor. The cast of the opposite (normal)

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*Ward's Natural Science Establishment, Rochester, N.Y.

Figure 7: Vinylite vascular cast of arteries and veins of a rat hind limb bearing a seven day old Walker 256 carcinoma.

(arrow marks tumor location)

Figure 8: Vinylite vascular cast of normal hind limb.

leg (Figure 8) reveals no such deficiency.

This marked absence of vessels in the region occupied by the tumor demonstrates that the tumor is relatively avascular, and confirms that the tumor is supplied only by small vessels from the periphery. The presence of a "hole" of this size in the vascular pattern of the leg cannot be explained by the small amount of central necrosis present in a six day old Walker tumor, nor by a lack of filling of small vessels in this area, as they are adequately filled in other parts of the limb. This defect in the vascular cast must therefore indicate the relatively avascular nature of this tumor.

The information provided by vascular casting techniques is admittedly of a gross nature and does not permit precise comparisons between the blood flows of different regions. In the normal physiological state, the majority of vessels present in the cast (11, 77) probably do not participate in the regional circulation at any given instant, and therefore, the presence or absence of vessels and their relative caliber is not direct evidence as to the magnitude of the blood flow to any region of the body.

C. Radio-autography of tumor vessels.

Introduction:

The previous studies of tumor vasculature utilizing Arteriography (page 3) and Injection-corrosion techniques (page 6) have supported the work of other investigators (5, 8, 9, 14, 29) indicating that solid tumors receive their blood supply from small vessels entering the tumor circumferentially from the periphery. This hypothesis can be further tested by the use of ceramic microspheres bearing a radioactive label (25, 30, 43, 74) which are introduced into the arterial supply to the tumor, with subsequent radio-autography to demonstrate microsphere location.

These microspheres are of such a diameter (25 $\pm$ 5 microns), that they will not pass through a capillary bed but become impacted in the smaller vessels. Assuming that these microspheres are completely mixed in the arterial blood at the level of the tumor and that they become impacted in the finer branches of the microcirculation, then their distribution will represent the distribution of arterial bloodflow to these areas at the time of arrival of the microspheres (25).

It must be emphasized that although the microspheres initially label the arterial blood, their trapping in the

capillary bed results in their distribution representing the distribution of arterial bloodflow at the time of the trapping.

Method:

Ceramic microspheres of 25 ± 5 microns diameter tagged with Scandium 46* were infused as a sudden intra-aortic injection into thirty rats bearing a nine day old Walker 256 carcinoma in the right gastrocnemius muscle. The injections were made into the thoracic aorta of animals lightly anesthetized with diethyl ether through a PE90 polyethylene cannula placed caudad in the right common carotid artery. Following the infusion the rats were sacrificed and the tumor bearing limb was amputated and fixed with 10% Formalin for forty-eight hours. A thick cross section of the tumor bearing area (Figure 9) was prepared and placed upon Kodak Xray film for an exposure time of forty-eight hours. The developed film (Figure 10) demonstrates the location of the microspheres in the tissue section.

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*Minnesota Mining and Manufacturing Company,
900 Bush Avenue,
St. Paul, Minn.

Figure 9: Partial cross-section of a rat hind limb bearing a nine day old Walker 256 carcinoma.

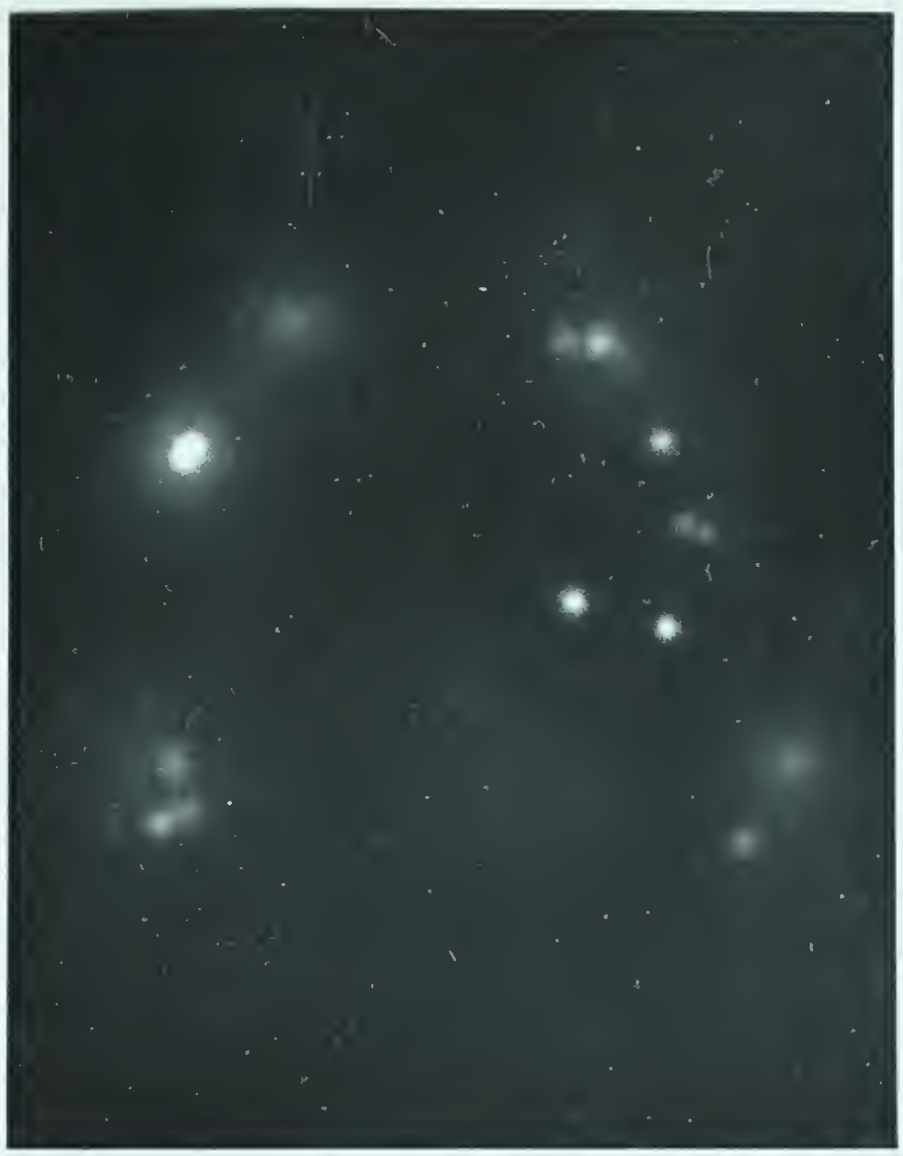

Figure 10: Radio-autogram of figure 9 (see text).

Results and Discussion:

The microspheres are seen to be distributed around the periphery of the tumor (Figure 10). In no tumor was there evidence of microspheres in the central areas of the tumor. The tumor therefore is not penetrated by arterial vessels of greater than 25 ± 5 microns in diameter.

The small size and peripheral location of the tumor arterial supply implies that nutrients or antitumor chemotherapeutic agents have a decreased access to the interior of the tumor. These features of tumor bloodflow may explain the central necrosis commonly observed in rapidly growing solid tumors, and may also be a factor in the disappointing clinical results achieved by antitumor chemotherapy.

D. Indicator-Dilution Techniques for Measuring Bloodflow

The measurement of bloodflow is an important part of many experimental studies, and there have been developed a number of physical and chemical methods for the determination of regional bloodflow (48, 59).

The physical methods utilize either direct reading meters which require the cannulation of regional vessels, or exterior appliances such as the rheograph (59) or the electromagnetic flowmeter which require some dissection of

these vessels. These physical methods have two major disadvantages, namely, the dissection or cannulation of the regional vessels probably produces some disruption of the normal physiology of the regional vascular bed; and secondly, these methods are not suitable for determining the bloodflow to small areas of tissue where arterial or venous vessels are not accessible.

The development of indicator-dilution methods in 1921 by Stewart (65) enabled estimations to be made of regional bloowflow without any disturbance of regional vasculature. More sophisticated adaptations of these methods (12, 16, 31, 33, 41, 42) provide for the estimation of bloodflow to small areas of the regional vasculature bed whose arterial and venous vessels are inaccessible for cannulation or sampling.

This study of the bloodflow to the Walker 256 carcinoma will of necessity utilize some of the latter methods.

Theory:

An indicator, as used in this sense, is a substance which permits the identification of some element of volume under study; showing the position of that element in space and with respect to time, and distinguishes the indicated element from all other elements of volume.

In these methods, a known quantity of indicator is introduced into a native fluid flowing at a unknown rate through a system of unknown volume. Fluid is sampled at one or more points downstream from the plane of injection, and the concentration of the indicator, diluted by the native fluid is determined and expressed as a function of time. It is claimed that from a knowledge of the mass of indicator injected and the sample concentration-time factor, the flow rate of the native fluid and the volume of the system can be determined. For the purpose of this discussion, only the flow rate will be discussed.

In the simplest form of these methods, when an indicator is introduced into a system at a constant rate in units of mass per time, and if mixing at the inflow is complete, the concentration of the indicator admitted to the system is:

$$\frac{\text{mass injected per time}}{\text{flow per time}} = \text{concentration} \qquad (1)$$

At the downstream sampling site, again assuming complete mixing, the concentration of indicator will rise from zero to reach a maximum concentration (equation 1). This maximum concentration, when reached, will allow measurement of the unknown flow rate, as given in the following equation:

$$\text{Flow} = \frac{\text{mass injected per time}}{\text{Maximum sample concentration}} \qquad (2)$$

Equation 2 requires only that mixing on indicator in native fluid is complete, and that there is no recirculation of indicator.

The ideal system for the study of these techniques has been defined as follows (76):

1. The system has a single inflow and a single outflow orifice through which all fluid in the system must pass.

2. Recirculation of indicator particles does not occur, that is, each unit of fluid upon leaving the system does not re-enter it.

3. Flow and volume must remain constant during the period of measurement.

4. The system must exhibit stationarity. This means that the indicator particles must have a distribution of transit times between entrance and sampling points which remains constant over the period of the experiment.

5. Finally, it is essential that the distribution of transit times of indicator particles be identical with those of the native fluid, that is, indicator and native fluid must mix thoroughly at the entrance to the system, and must remain mixed throughout the period of the experiment.

Real vascular systems violate the conditions proposed for the ideal system, and these violations affect the validity of these methods for the measurement of flow:

1. The system does not have a single inflow and outflow orifice. This violation is not important if the system includes a single channel through which all particles of fluid must pass and in which mixing can take place. In such a case, flow can be measured by the equations shown earlier (1 and 2) as equation 1 requires only that all indicator must leave the system and that during some time interval prior to sampling all indicator must be diluted by a volume equal to the flow in that time interval; and equation 2 depends upon the fact that once the injected indicator has been diluted so that its concentration is the injected mass divided by flow, it can neither be concentrated further nor diluted, and therefore the outflow concentration must eventually become constant at the injected mass divided by the flow.

2. Recirculation of indicator particles does occur. This violation is important only if recirculation occurs before all indicator particles have completed the first transit, confusing the interpretation of the primary transit curve. Problems created by recirculation may be treated in a variety of ways (16, 23). In the methods used in

these experiments recirculation is unimportant, as will be discussed in a later section.

3. Flow or volume or both are not constant. If the rate of change in flow is slow when compared to the average transit times of the indicator particles, or if the change in flow is from one steady state to another, then the limiting concentration (equation 2) will accurately reflect changes in flow, provided that the indicator is being introduced into the system at a constant rate. Methods involving a sudden single injection of indicator are not possible in such cases.

4. The system is non stationary--the assumption of a constant distribution of transit times for indicator particles is certainly violated in pulsatile vascular systems. However, if the phasic changes in transit times are brief in relation to the period of measurement as in the case of vascular systems, then these violations are not important.

5. The flow of indicator particles is not representative of the native fluid. Selection of the appropriate indicator is the responsibility of the investigator and is difficult in the case of a heterogenous native fluid such as blood coursing through a leaky system such as the vascular system. If the chosen indicator is nondiffusible

(does not leave the vascular system), then the concentration of indicator in the capillary is greater than the limiting concentration (equation 1) due to net losses of water in the arteriole end of the capillary. If the indicator is diffusible and is not all returned to the vascular system, then the flow which is measured is that which originally diluted the indicator only if the indicator is admitted to the system at a constant rate.

The bloodflow carried to an area of the body by the regional circulation consists of two separate volumes. There is that portion of the regional flow which is shunted around the capillary bed to enter the venous drainage of the area through arteriovenous shunt vessels and therefore does not participate in blood-tissue exchange. This shunt flow is present to a greater or lesser degree in all organs and tissues and varies considerably in magnitude according to local conditions (11, 77).

The second fraction, usually the major fraction of the regional or organ bloodflow passes through the capillary bed of the tissues. It is this fraction which carries the nutrients (or chemotherapeutic agents) to the tissue cells and which transports away the wastes. For our purpose, this fraction will be called the tissue bloodflow as distinguished from the shunt flow.

External (physical) measurements of regional bloodflow or indicator dilution methods employing nondiffusible indicators and depending upon venous sampling must necessarily measure the total regional flow, that is, shunt plus tissue bloodflow. Estimations of tissue bloodflow to small areas of the body are most easily accomplished by a further adaptation of the general principle of indicator dilution methods outlined previously. The derivation of these methods is as follows.

It can be shown (41) that for any substance carried by the flow of blood to a region or organ under examination within a stipulated time interval (dt), the quantity delivered ($dQa$) must equal the quantity disposed of by accumulation ($dQi$), by metabolic conversion ($dQm$), or by transport out of the region ($dQe$) by all routes of egress, or:

$$\frac{dQa}{dt} = \frac{dQi}{dt} + \frac{dQm}{dt} + \frac{dQe}{dt} \qquad (3)$$

Assuming that the indicator substance is not metabolized, or is metabolized to an insignificant degree in the time interval chosen, $dQm$ becomes zero, and the equation becomes:

$$\frac{dQa}{dt} = \frac{dQi}{dt} + \frac{dQe}{dt} \qquad (4)$$

Combining equations 2 and 4 and expressing them in the integral form yields the general form of the Fick principle:

The flow to a region (in units of volume/volume tissue/time) is equal to the tissue concentration of the indicator substance (in units of volume/volume) divided by the sum of the arterial minus the venous concentrations expressed as a function of time, or:

$$\text{Flow (ml/min/gm)} = \frac{\text{tissue concentration}}{\int (\text{Cart.} - \text{Cv})\, dt} \qquad (5)$$

If the arterial sample is collected at a constant rate, this method of sampling will provide an automatic integration with respect to time. Therefore, the mean arterial concentration may be substituted for the function $\int Ca\,dt$, and the equation becomes:

$$\text{Flow} = \frac{\text{tissue concentration}}{(\text{mean Ca} - \int Cv\,dt)} \qquad (6)$$

The determination of regional bloodflow to each small component of tissue using either equation 5 or 6 involves the measurement of the venous concentration of indicator leaving each of these small components of tissue. The collection of this venous sample will present a formidable technical problem as the sample must be from only that venous blood draining the entire tissue sample and

cannot be contaminated by venous blood draining from other adjacent tissue areas. This difficulty is avoided by substituting the estimation of the venous integral proposed by Johnson (33), where the integral $\int Cvdt$ is estimated by the function $\frac{\text{tissue concentration x time}}{2}$, and the form becomes:

$$\text{Flow} = \frac{\text{Tissue concentration}}{(\text{mean art. conc.} - \frac{\text{tissue conc.}}{2})t} \quad (7)$$

The estimation of the venous concentration integral, $\int Cvdt$, by the function (tissue conc./2)t (33, 53, 61) depends upon the assumption that the venous concentration rose from zero at time zero in a linear function to equal tissue concentration at time T. Johnson (33) compared calculated flows assuming several different venous conc./time curves and found that the flow rates were changed by only 5-10% if the time interval was sufficiently short to prevent tissue and arterial blood concentrations from reaching equilibrium. He also found that when venous conc./time curves were determined for a whole organ, the resultant curve tended to be sigmoid and could be reasonably approximated by a straight line.

The utilization of equation 7 to calculate tissue flows involves several other assumptions based upon the indicator substance. The indicator chosen must be freely

diffusible, must be either not metabolized or metabolized to an insignificant degree in the time interval chosen; the tissue-blood partition coefficient for this indicator must approximate unity and tissue-arterial blood equilibrium with respect to indicator concentrations must not take place within the time interval chosen. These assumptions will be evaluated under the heading Discussion.

Methods:

The indicator chosen for the following series of experiments was 4-Iodoantipyrine $I_{131}$ (Abbott). This indicator has a number of useful properties for such techniques as will be outlined under the heading Discussion.

The tumor preparation used was the standard preparation as outlined on page two. Briefly, this is a four to six day old Walker 256 carcinoma implanted in the right hind limb musculature of Sprague-Dawley rats weighing between 250 and 350 grams.

A. Intra-venous Infusion.

The indicator was delivered to the rat at a constant rate by a Harvard model 600-930 syringe pump delivering approximately five micro-curies of Iodoantipyrine to the left femoral vein of the rat via a PE90 polyethylene cannula over a time interval of seventy seconds. The time interval was timed from the entry of the column of

Iodoantipyrine into the vein of the animal. Arterial samples were collected from the left femoral artery of the animal at a constant rate employing the withdrawal side of the same pump (Figure 11). The infused volume was the same as that of the arterial sample withdrawn, to minimize any fall in arterial pressure consequent to sample withdrawal. The arterial pressure in the experimental animal was not monitored as there was no significant change in carotid pressure in a pilot group of ten rats where the arterial pressure (carotid)averaged 91 mm Hg.

The time interval was recorded on a stopwatch. At the termination of the interval, sample collection was ceased, the right hind limb (tumor-bearing) was rapidly amputated, with heavy shears, and samples of tumor, skeletal muscle and skin of approximately one gram weight were removed and placed in individual weighed stoppered tubes. These samples were then weighed to a tolerance of 0.1 mg. and the radioactive content of the samples was determined by counting the samples individually for five minutes in a well type scintillation counter. Tissue and arterial sample concentrations of indicator were calculated as counts per minute per gram, and the blood flows were calculated as in equation 7 (p. 20).

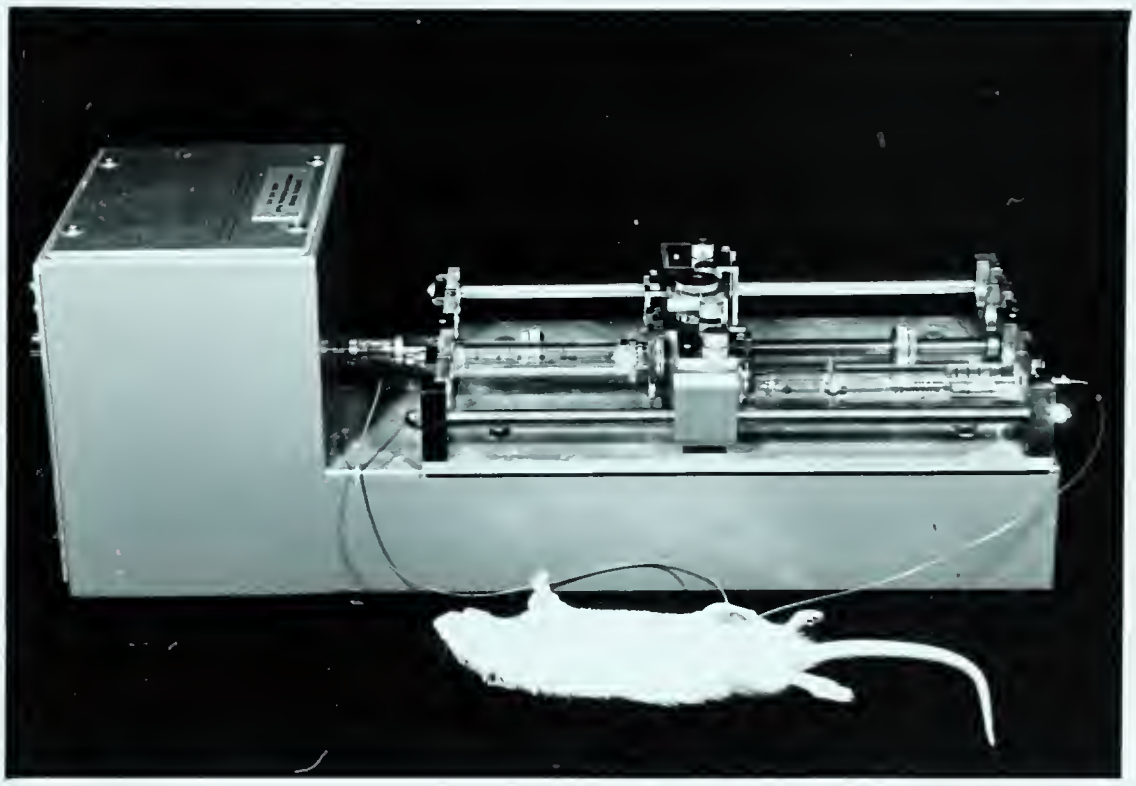

Figure 11: Constant rate intravenous infusion method for the determination of tissue bloodflows: Apparatus.

B. Isolated Femoral Artery Perfusion Techniques.

The isolated femoral artery perfusion technique developed by Couves and co-workers (68) was utilized to determine tissue bloodflows under conditions utilized for regional chemotherapy of tumors. The isolated tumor bearing leg provides a satisfactory preparation for the comparison of bloodflow to the capillary beds of the various tissues of the limb, as the indicator will be distributed to the tissues of the leg in proportion to their bloodflow. (infra and 30, 55, 56). This preparation also provides a simple means for the determination of the venous-concentration/time curve for the entire leg.

The apparatus (Figure 12) is an adaptation of that developed by Couves (68). The femoral vessels of the tumor bearing limb are dissected free and cannulated with PE90 polyethylene tubing, the end of which has been drawn to a smaller diameter over a low Bunsen flame. Isolation of the hind limb is provided by the use of a rubber band tourniquet tied around the limb root, passing beneath the femoral vessels. The circulation to the limb is thereby restricted chiefly to that through the femoral vessels. The degree of indicator leak into the general circulation of the animal was determined in each case by withdrawal of a venous sample from the animal at the conclusion of the

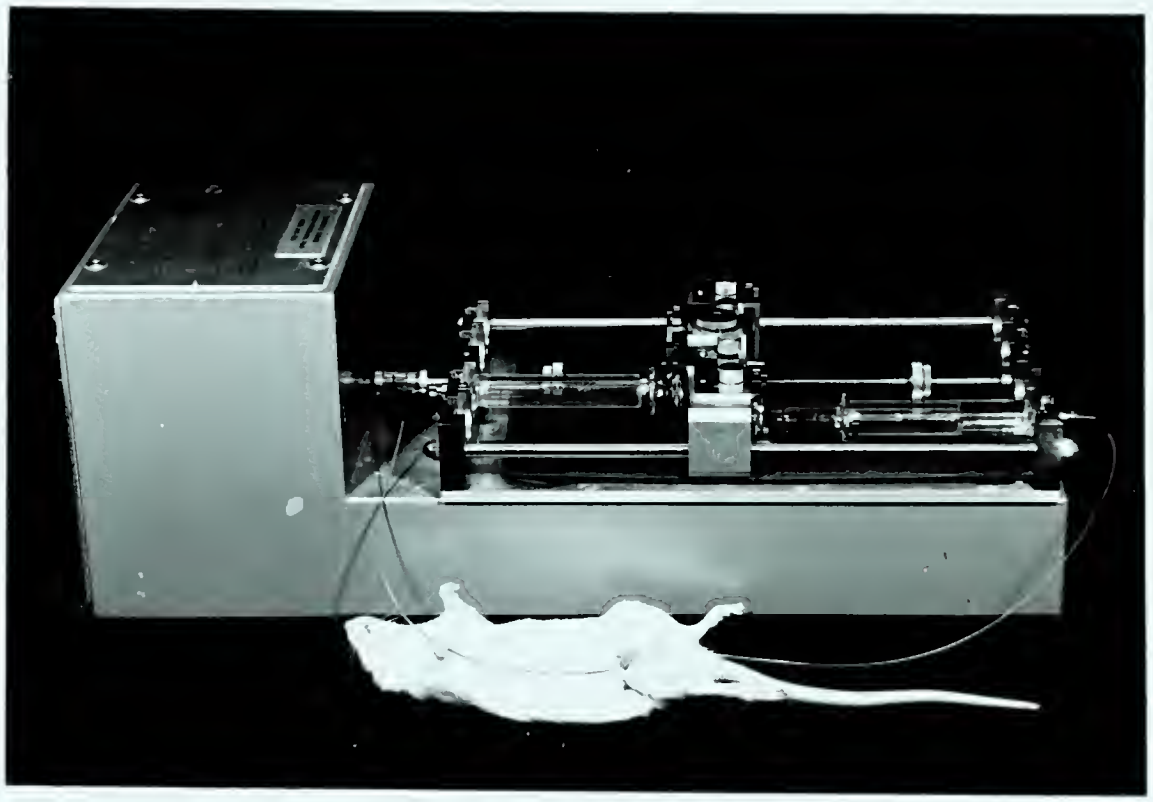

Figure 12: Isolated perfusion method for the determination of tissue bloodflows: Apparatus.

experiment and the determination of its radioactive content. Animals showing leakage were discarded. No determination of leak in the opposite direction was made, that is, leak from the animal's general arterial circulation into the leg.

Approximately five micro-curies of Iodoantipyrine was dissolved in heparinized blood from a donor rat and administered to the animal at a constant rate of approximately 1.0 ml. per minute for a sixty second interval. The time zero was taken as the point where the column of indicator from the pump entered the right femoral artery. Sampling of the venous effluent from the leg was accomplished through the withdrawal side of the same syringe pump (Figure 12).

At the termination of the sixty second interval the pump was stopped, and samples were removed from the skin, tumor, and muscle of the isolated leg. Samples were handled as before: weighed to the nearest 0.1 mg. and counted for five minutes is a well type scintillation counter. Samples of the venous blood from the animal's inferior vena cava, as described above, were taken to provide a control of the assumption that the limb is isolated from the general circulation.

Results:

TABLE I

INFUSION TECHNIQUE: RESULTS

| TISSUE | GROUP SIZE | MEAN FLOW (ml/min/gm) | STANDARD DEVIATION | RANGE |
|---|---|---|---|---|
| Muscle | 56 | 0.38 | ± 0.008 | 0.23-0.71 |
| Tumor | 56 | 0.27 | ± 0.009 | 0.15-0.41 |
| Skin | 56 | 0.23 | ± 0.008 | 0.13-0.41 |

The magnitude of tumor bloodflow in the rat anesthetized with Pentobarbital sodium (30 mg/kg), as measured by this method, is significantly less than the flow to resting skeletal muscle and only slightly higher than that to the skin.

TABLE II

PERFUSION TECHNIQUE: RESULTS

| TISSUE | GROUP SIZE (no. rats) | MEAN FLOW (ml/min/gm) | STANDARD DEVIATION | RANGE |
|---|---|---|---|---|
| Muscle | 30 | 0.44 | ± 0.008 | 0.37-0.51 |
| Tumor | 30 | 0.26 | ± 0.007 | 0.18-0.31 |
| Skin | 30 | 0.24 | ± 0.003 | 0.19-0.30 |

Tumor bloodflow under conditions of isolated femoral artery perfusion is less than the bloodflow to skeletal

muscle in the same limb.

Discussion:

The validity of the estimations of tissue bloodflow provided by the methods used in this study depend upon a number of assumptions regarding the method and the indicator chosen. Those general assumptions involving indicator-dilution methods were discussed under the heading Theory (supra). The assumptions involving the indicator and the sources of error in the chosen experimental method will be considered at this point.

A freely diffusible non-metabolized substance carried to a region by the blood will reach a concentration (X) in each small component of tissue (i) in time (T) which depends upon concentration of the substance in the arterial blood (Ca) during that period of time and upon local conditions of capillary bloodflow per unit of tissue volume (Fi/Vi), tissue-blood partition coefficient of the substance (Zi), and the effectiveness of tissue-capillary diffusion (Mi) which is obtained in each small component in the time interval chosen. This may be expressed mathematically (42):

$$Xi(T) = ZiKie^{-KiT} \int_0^T Cae^{KT} dt \qquad (8)$$

where $Ki = MiFi/ZiVi$

The tissue bloodflow through a small component of

tissue may be calculated from equation (8) if these quantities are known or may be measured.

The method used in this study and the equation (number seven, page 20) employed to calculate the results are adapted from the above expression. The calculation of the results assumes that the tissue blood partition coefficient for iodoantipyrine is unity. Kety (42) determined the partition coefficient for this indicator in the rat in experiments in which (after hepatectomy to prevent metabolization) the arterial blood concentration was held constant and the equilibration period was lengthened to one hour. He found that the values of Zi in various tissues was reasonably constant and equal to unity.

Using a mathematical method (42) Kety calculated values of K from a curve of Ca/T and measurements of XiT and Zi, and found that there is a linear relation between tissue concentrations of Iodoantipyrine and bloodflow. The tissue concentrations of this indicator are therefore limited by bloodflow and not by diffusion, thus satisfying another important assumption regarding iodoantipyrine, namely, that it is freely diffusible. The diffusibility of iodoantipyrine and flow-limited nature of the tissue distribution of this indicator have been established by other investigators. Johnson (33) compared the flow rates

determined in whole body perfusions of dogs using iodoantipyrine with those obtained using $D_2O$ as the indicator and found close agreement. $D_2O$ is well established as a flow-limited substance (32, 33, 52, 66).

Iodoantipyrine is slowly metabolized in the liver, at a rate averaging 6% per hour in the human and the dog (10). Presumably the rate in the rat is of a similar low order. This extent of metabolic degradation is not significant in experiments lasting sixty or seventy seconds.

From the foregoing, it is likely that our assumptions involving the indicator with respect to diffusion rate, extent of diffusion equilibrium and tissue-blood partition coefficient are justified and will introduce no significant error into these determinations.

A related source of error involving the indicator in comparing bloodflows between different tissues is the assumption that each tissue has an identical ability to remove iodoantipyrine from the diffusible portion of the vascular bed, that is, that the extraction ratios for this substance are the same from tissue to tissue. While the investigations of Kety (42) and of Johnson (33) would appear to satisfy this assumption, a gross check may be applied to show differences in extraction of iodoantipyrine by the tissues used in this series of experiments.

The distribution of a diffusible substance to the tissues of the body after a single sudden injection will be in proportion to the fraction of the cardiac output received by that tissue. The persistence of that substance in the various tissues after this single injection will be related to factors involving the diffusion and the extraction ratios of each particular tissue for that substance (55, 56).

Comparisons of the persistence of 4-Iodoantipyrine content in muscle, tumor and skin are given in Table III and Figure 13. These were determined as the mean values of five rats in each numerical group. Sprague-Dawley rats bearing a five day old Walker 256 tumor were given a single injection of approximately 10 micro-curies of iodoantipyrine into the left femoral vein at time zero and the tumor-bearing limbs were rapidly amputated with heavy shears after the time interval indicated. Samples of tumor, muscle and skin were removed and the indicator concentration was determined in the manner previously described (page 21).


Figure 13: The persistence of 4-Iodoantipyrine in various tissues following a single rapid intravenous infusion.

TABLE III

PERSISTENCE OF 4-IODOANTIPYRINE IN TISSUES FOLLOWING A SINGLE RAPID IV DOSE

| TIME INTERVAL (seconds) | TISSUE CONCENTRATION (cpm/gm) Tumor | Muscle | Skin |
|---|---|---|---|
| 0-10 | 58 | 72 | 23 |
| -20 | 485 | 883 | 174 |
| -30 | 788 | 2747 | 868 |
| -40 | 734 | 2895 | 701 |
| -50 | 1253 | 3444 | 1145 |
| -60 | 1617 | 2480 | 1492 |
| -70 | 1983 | 2634 | 1008 |
| -80 | 987 | 2302 | 879 |
| -90 | 649 | 2451 | 443 |
| -100 | 897 | 2352 | 533 |
| -110 | 993 | 2270 | 583 |

The similar shape of the curves (Figure 13) indicate that the factors operating in each tissue are similar and no gross error is introduced by the assumption that diffusion or persistence of the indicator is the same in these tissues when comparing bloodflow determinations.

The justification of these assumptions involving the indicator as described in equation 8 (page 26), permits the use of the considerably simpler form of equations 6 and 7 (pages 19, 20), which are sufficiently accurate for the calculation of bloodflow in these tissues (tumor, muscle and skin) using this indicator (4-Iodoantipyrine).

It must be emphasized that rate, extent and uniformity of diffusion of the indicator are significant in an

experimental method such as this, where venous concentrations leaving the tissue sample are not measured, and where the assumption of rapid equilibration between capillary and tissue concentrations is made (equation 7, page 20). In such cases, as has been shown by Kety (41, 42) and others (33, 52, 57, 61), appropriate theoretic or experimental evidence is necessary to justify this assumption in every case.

In addition to those sources of error involving properties of the chosen indicator substance, there are a second group of errors arising from the particular experimental method and calculations of bloodflow which were employed.

The adoption of mean arterial concentration as a substitute for $\int Cadt$ (equation 6, page 19) requires that the concentration-time curve for arterial blood be approximated by a straight line although errors introduced by a skew of the curve would be slight. The shape of the arterial concentration vs. time curve in the constant rate intravenous infusion preparation was determined in the following experiment:

Fifteen Sprague-Dawley rats were given 4-Iodoantipyrine as a constant rate infusion from a Harvard 600-930 syringe pump into a femoral vein cannula. Timed samples

were collected from the left femoral artery by manually moving a collecting cannula from one sample tube to the next (Figure 14). The time interval was eighty seconds. The samples of arterial blood were weighed to the nearest 0.1 mg and radioactivity was determined by counting in a well type scintillation counter for five minutes. Concentrations were expressed in counts per minute per gram of sample (Figure 15, Table IV).

TABLE IV

ARTERIAL CONCENTRATION VERSUS TIME

| TIME (seconds) | ARTERIAL CONCENTRATION (cpm/gm) |
|---|---|
| 0-10 | 996 |
| 20 | 10945 |
| 30 | 15830 |
| 40 | 19195 |
| 50 | 21206 |
| 60 | 23011 |
| 70 | 25131 |
| 80 | 26059 |

Following the intravenous administration of Iodoantipyrine, there is approximately a ten second interval before the indicator appears in the arterial blood in significant concentrations. This time lag dictated the use of a seventy second time interval in the experimental method (page 21). In the calculations this time lag was applied as a correction both to the time interval

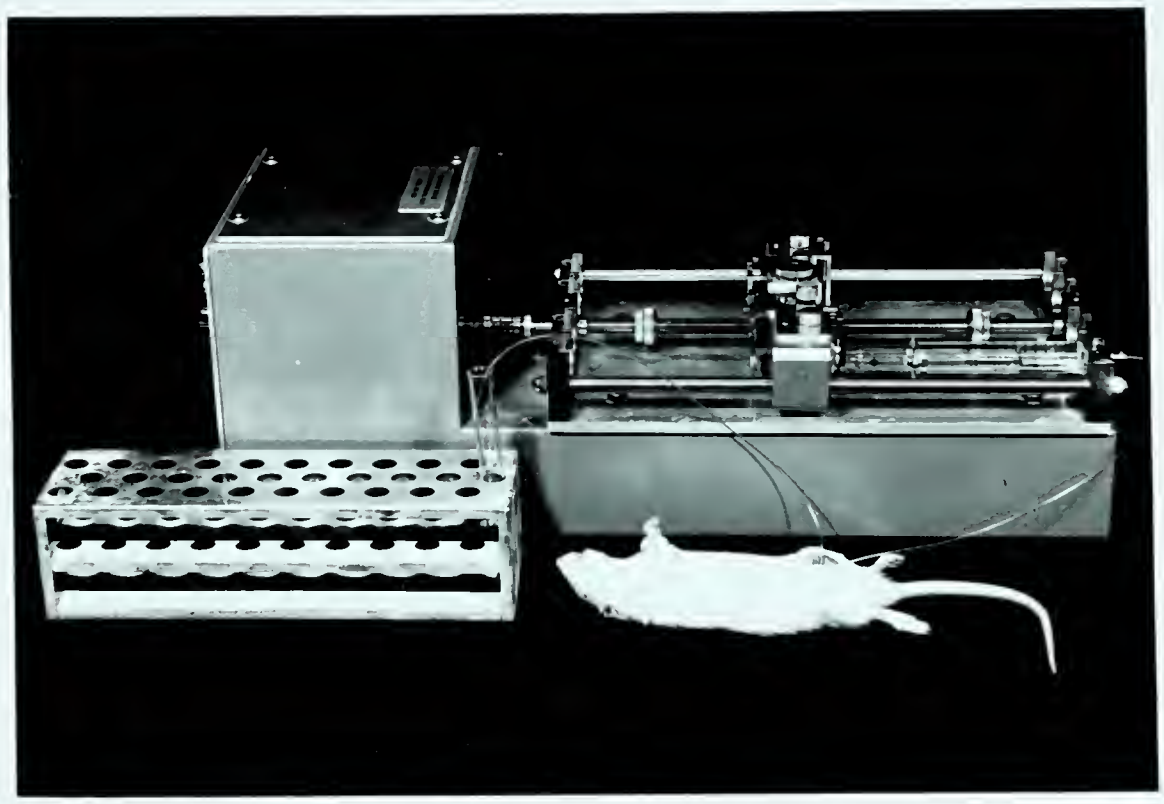

Figure 14: Infusion technique: Collection of arterial samples for the determination of Ca/T.

(60 seconds was taken as T), and to the mean arterial concentrations which were increased by a factor of 7/6 to allow for the collection of sample before the appearance of indicator in the arterial blood.

The arterial concentration-time curve (Figure 15) can be reasonably approximated by a straight line once this initial lag is accounted for. This curve agrees with the findings of Kety (42), who demonstrated an almost linear relationship between arterial concentrations and time when using a similar preparation and this same indicator.

The calculation of tissue bloodflows by means of equation 7 (page 20) introduces an additional error through the use of the estimate of the venous concentration/time integral $\int Cvdt$ which was proposed by Johnson and co-workers (33). Calculations of tissue bloodflow by indicator-dilution methods depending upon the collection of venous samples, such as:

$$\text{Flow} = \frac{\text{Tissue concentration}}{\int (Ca-Cv)dt}$$

where tissue concentrations are determined from small samples are valid only if the venous sample is collected from the venous drainage of the region of the sample without contamination by blood draining from other adjacent

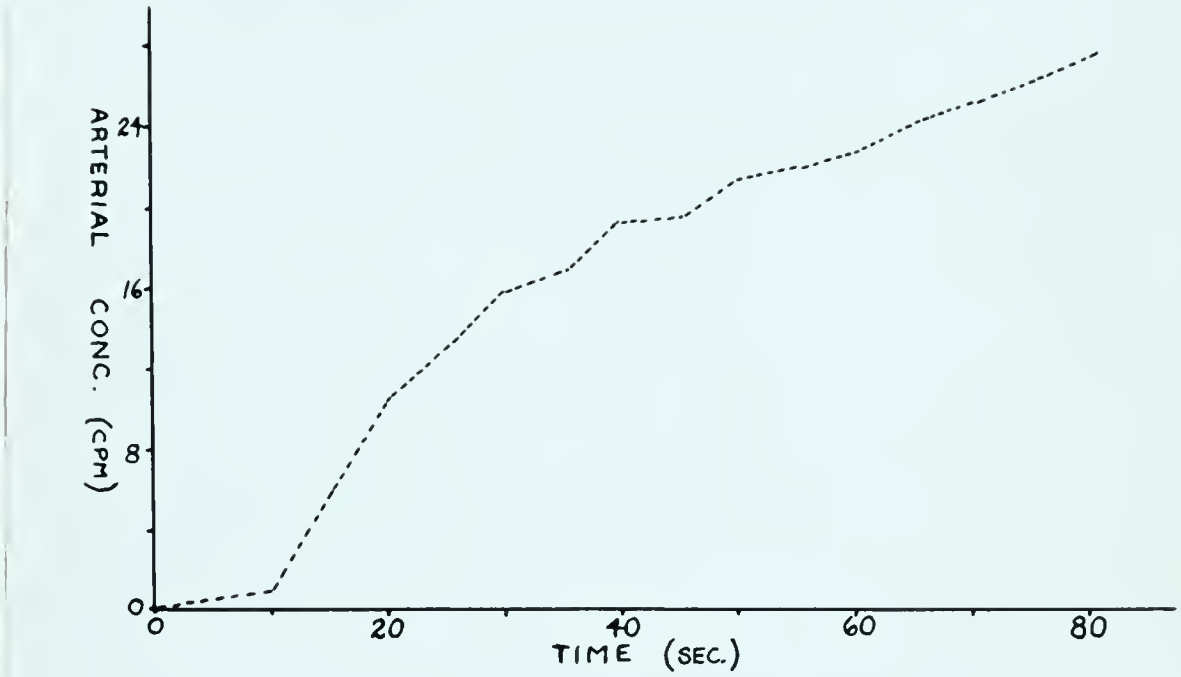


Figure 15: Arterial concentration of 4-Iodoantipyrine versus Time--infusion technique.

tissue areas; due to the heterogeneity of tissue perfusion within all organs and tissues (11, 77). These errors may be minimized by taking tissue samples of the largest practical size.

The collection of uncontaminated venous samples from small tissue samples is an impossible technical problem, and therefore Johnson and co-workers (33) proposed the following estimate for the integral $\int Cvdt$.

They proposed that $\int Cvdt$ may be approximated by the function tissue concentration/2 x Time, assuming that the venous concentration rises linearly from zero at time zero to equal the tissue concentration at time T. Thus, the venous concentration/time curve must be approximately a linear function, tissue-capillary blood equilibrium must occur within time T, and the time interval must be sufficiently short that arterial blood-tissue concentration-equilibrium does not occur.

The preceding discussion of diffusion rates of this indicator (4-Iodoantipyrine) has shown that capillary blood-tissue diffusion is not a limiting factor. In our experiments, the arterial concentrations were four to ten times greater than the tissue concentrations, hence arterial blood-tissue concentrations had not reached a state of equilibrium. The assumption that the venous concentrations/

time curve rises in a linear fashion requires further consideration. This assumption will not produce any significant error in the determinations, as has been shown by Johnson (33), insofar that the shape of the curve will produce relatively small changes in the magnitude of $\int Cvdt$. The following experiment was devised to determine the shape of the venous concentration/time curve for the entire hind limb. This curve is not truly valid for the curve from each small tissue component, but should be a closely related curve and will indicate any gross error in the assumption of a linear relationship. Johnson calculated the value of $\int Cvdt$ for a widely different series of time courses of venous concentration and found that the value of $\int Cvdt$ was distributed over a range of only 5% (33).

Fifteen Sprague-Dawley rats were prepared for the isolated perfusion technique as described elsewhere (page 23). Iodoantipyrine in blood was infused through the femoral artery cannula of the isolated hind limb at a constant known rate, and multiple discrete samples were collected in tubes by an automatic sample collector at five second intervals. The concentration of Iodoantipyrine in these samples was determined as previously described and expressed as counts per minute per gram (cpm/gm). These concentrations were then plotted against time (Figure 17,

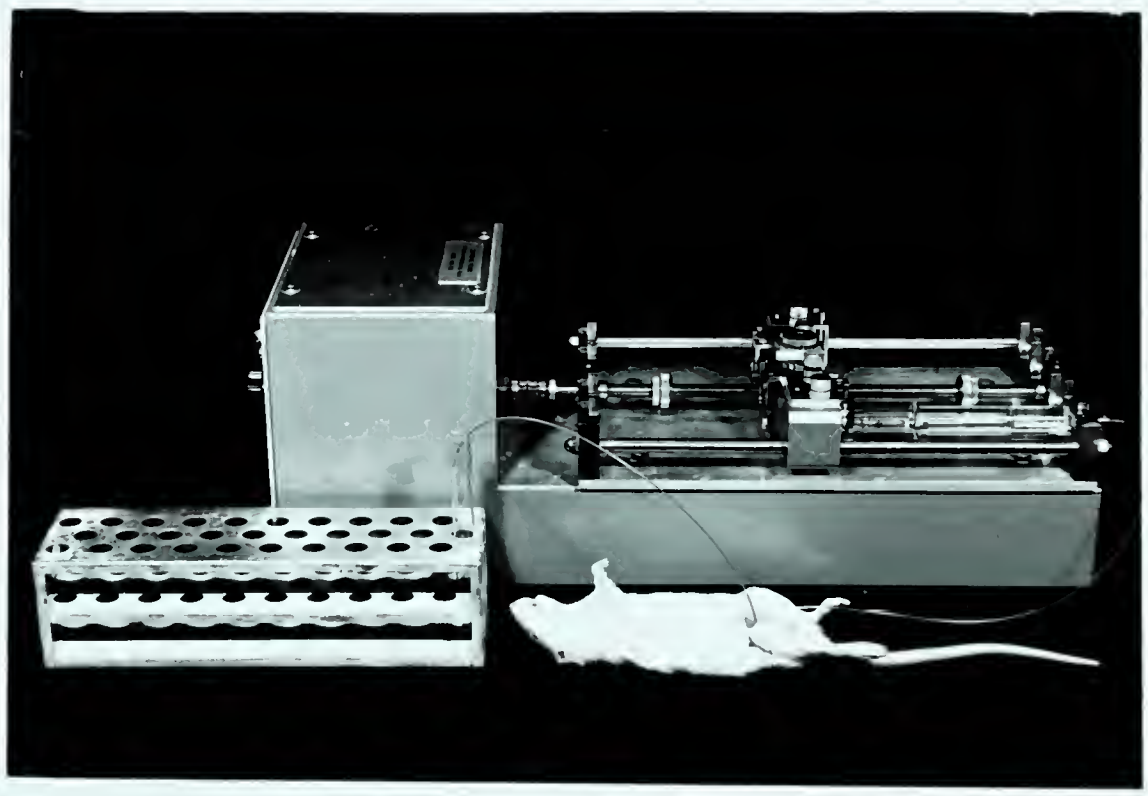

Figure 16: Perfusion technique: Collection of venous samples for the determination of Cv/T.

Table V).

TABLE V

VENOUS CONCENTRATIONS VERSUS TIME

| TIME (seconds) | VENOUS CONCENTRATIONS (cpm/gm) |
|---|---|
| 0-10 | 532 |
| -20 | 888 |
| -30 | 1675 |
| -40 | 4133 |
| -50 | 5894 |
| -60 | 8309 |
| -70 | 10664 |

The venous concentration/time curve for the entire hind limb (Figure 17) is of sigmoid shape and can reasonably be approximated by a straight line. Therefore, the use of this estimate for $\int Cvdt$ involves no serious error in the determination of tissue bloodflows and any error present will probably apply equally to all tissues of the limb.

In summary, the experimental method chosen, employing a diffusible indicator, 4-Iodoantipyrine $I_{131}$, and indicator dilution methods for the determination of tissue bloodflows to skin and muscle of the rat hind limb, and to the Walker 256 carcinoma implanted in the hind limb musculature, involves a number of theoretic sources of error. These potential errors are principally the assumptions

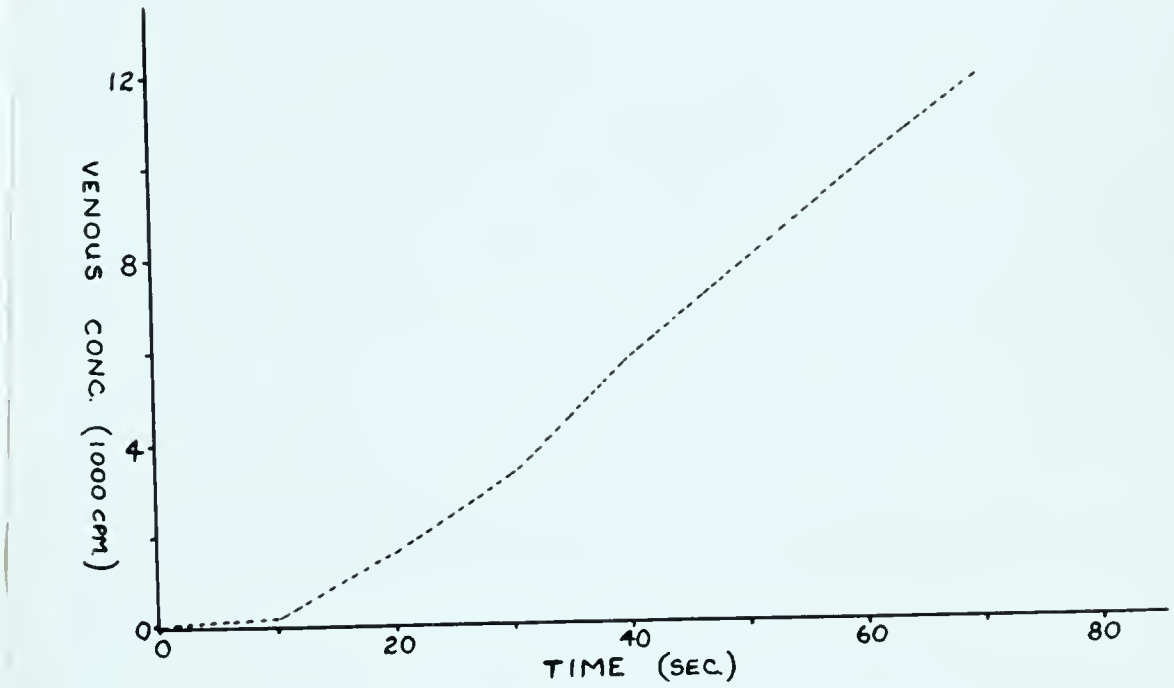


Figure 17: Venous concentration of 4-Iodoantipyrine versus Time. Composite curve for the entire hind limb--perfusion technique.

regarding the rate and extent of diffusion of the indicator and the time courses of the arterial and venous concentrations of indicator. These assumptions have been considered individually , both in theory and by experiment, and have been found to introduce errors of only small magnitude.

The magnitude of capillary bed (tissue) bloodflow in the tumor (0.27 ± 0.009 ml./min./gm.) as determined by this method was found to be considerably smaller than that of resting muscle (0.38 ± 0.008 ml./min./gm.) and only slightly higher than that of the skin (0.23 ± 0.008 ml./min./gm.).

## CHAPTER II

### THE BLOODFLOW TO THE NORMAL HIND LIMB

The presence of the tumor (Walker 256 carcinoma) implanted in the right hind limb musculature may have some effect on the bloodflows to the other tissues of the limb. This effect of the tumor may affect the validity of comparisons between the flow to the tumor and that to the other tissues of the leg.

This factor was investigated by the determination of tissues bloodflows to the tissues of the right hind limb in rats which do not bear the tumor, and comparison with those of tumor-bearing rats. The method was that of constant rate femoral vein infusion which was previously described (page 21).

Method:

Thirty Sprague-Dawley rats were lightly anesthetized with Pentobarbital sodium (30 mg/kg) through the intra-peritoneal route. The left femoral artery and vein were cannulated with PE90 polyethylene cannulas. Iodoantipyrine (approx. 10 microcuries) was infused at a constant rate into the left femoral vein for a period of seventy seconds, and simultaneous arterial sampling at a constant rate over this period was achieved. At the termination of the

period of infusion, the right hind limb was rapidly amputated with heavy shears. Samples of muscle and skin were taken from the usual sites and treated in the manner described on page 22. Sample concentrations of Iodoantipyrine and bloodflows were determined and are tabulated in Table VI.

Results:

TABLE VI

THE BLOODFLOW TO TISSUES OF THE RIGHT HIND LIMB IN NORMAL AND TUMOR BEARING ANIMALS

| TISSUE | GROUP SIZE | MEAN FLOW (ml/min/gm) | STANDARD DEVIATION | RANGE |
|---|---|---|---|---|
| Normal leg: | | | | |
| Muscle | 30 | 0.37 | ± 0.007 | 0.36-0.50 |
| Skin | 30 | 0.23 | ± 0.003 | 0.19-0.30 |
| * Tumor bearing leg: | | | | |
| Muscle | 56 | 0.38 | ± 0.008 | 0.23-0.71 |
| Skin | 56 | 0.23 | ± 0.008 | 0.13-0.41 |

* page 25.

Discussion and Conclusions:

The bloodflows to the muscle and skin in the tumor bearing animal are almost identical to those of the normal animal. Therefore the presence of the tumor has no significant effect on the flows to the other tissues. The similarity of the mean flows to the tissues of the right hind

limb under conditions of light Pentobarbital anesthesia in these two groups is remarkable.

# CHAPTER III

## THE BLOODFLOW TO THE WALKER 256 CARCINOMA IN OTHER SITES OF IMPLANTATION

The preceding chapters have been concerned with the magnitude and architecture of the blood supply to the Walker 256 carcinoma implanted in the hind limb musculature. It becomes important to determine whether the amount of bloodflow to the tumor is an inherent feature of the tumor, or whether this is a feature of the site of implantation. The bloodflow to the tumor was therefore investigated in two other tissues; as a renal implant, and as metastases from the hind limb tumor to the para-aortic lymph nodes.

### A. Bloodflow To the Renal Implant:

The tumor was implanted in the left kidney of twenty-five Sprague-Dawley rats as a tissue-isolated tumor using the method described by Gullino and Grantham (26). This preparation provides the kidney and tumor with a paraffin envelope which prevents the tumor from developing a blood supply from the adjacent tissues by invasion and therefore represents a tumor supplied with a single artery and a single vein (the left renal artery and vein respectively).

Method:

An hourglass shaped piece of Parafilm M* was constructed as shown in Figure 18. Under diethyl ether anesthesia, the left kidney of the rat was dissected free of the perirenal fat and brought to a subcutaneous position (Figure 19) through a left loin muscle-splitting incision. The choice of the left kidney is dictated by the longer vascular pedicle of this side when compared to the right kidney. The kidney was then enclosed in the folded paraffin sheet with 25 mg. of Chloramphenicol to discourage the development of infection, using a small pencil soldering iron to seal the edges of the envelope (Figure 20). The enclosed kidney was then returned to a subcutaneous pocket produced by undercutting the dorsal skin. The muscle layers were not approximated to prevent compromising the blood supply to the kidney, and the skin was closed with Michel slips (Figure 21).

Following a seven day interval, chosen to be certain that the vascular supply to the kidney was not impaired and that the preparation did not become infected, the skin wound was reopened under sterile precautions and

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* American Can Comp any,
Marathon Division,
Menaska, Wisc.

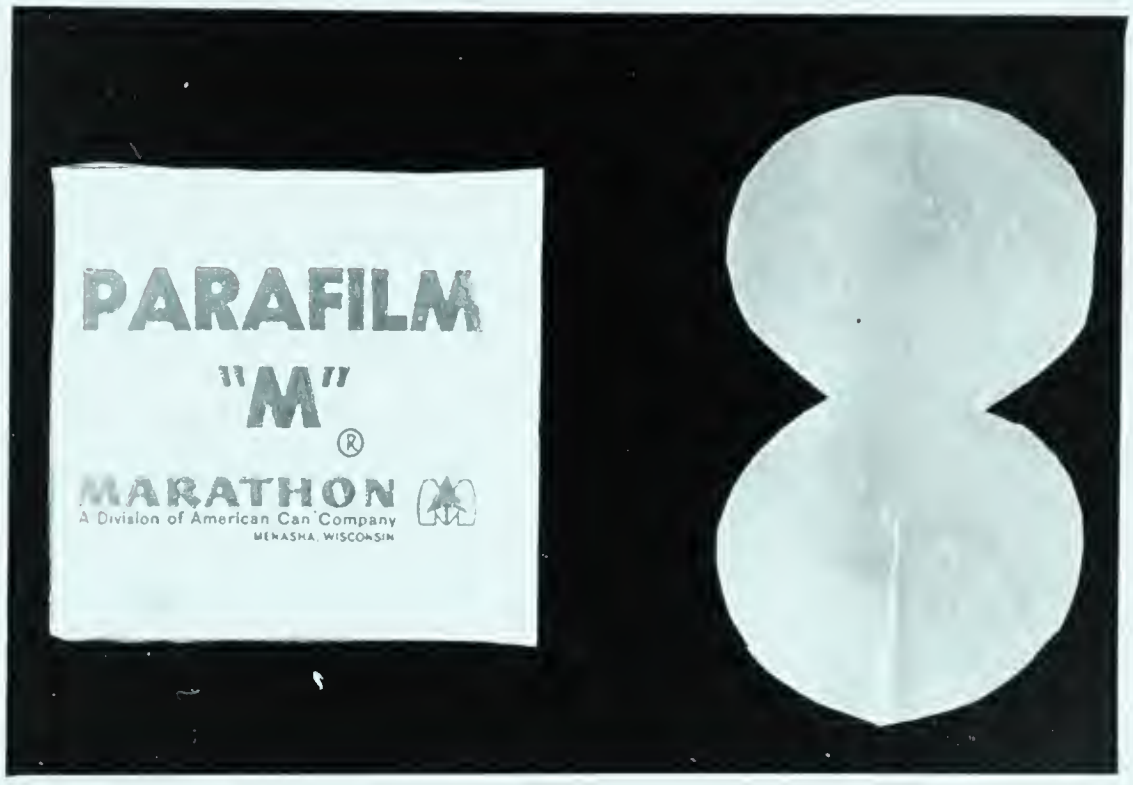


Figure 18: The tissue isolated tumor (26): Construction of the paraffin envelope.

Figure 19: The tissue isolated tumor (26): Dissection of the left kidney.

Figure 20: The tissue isolated tumor (26): Isolation of the left kidney in envelope.

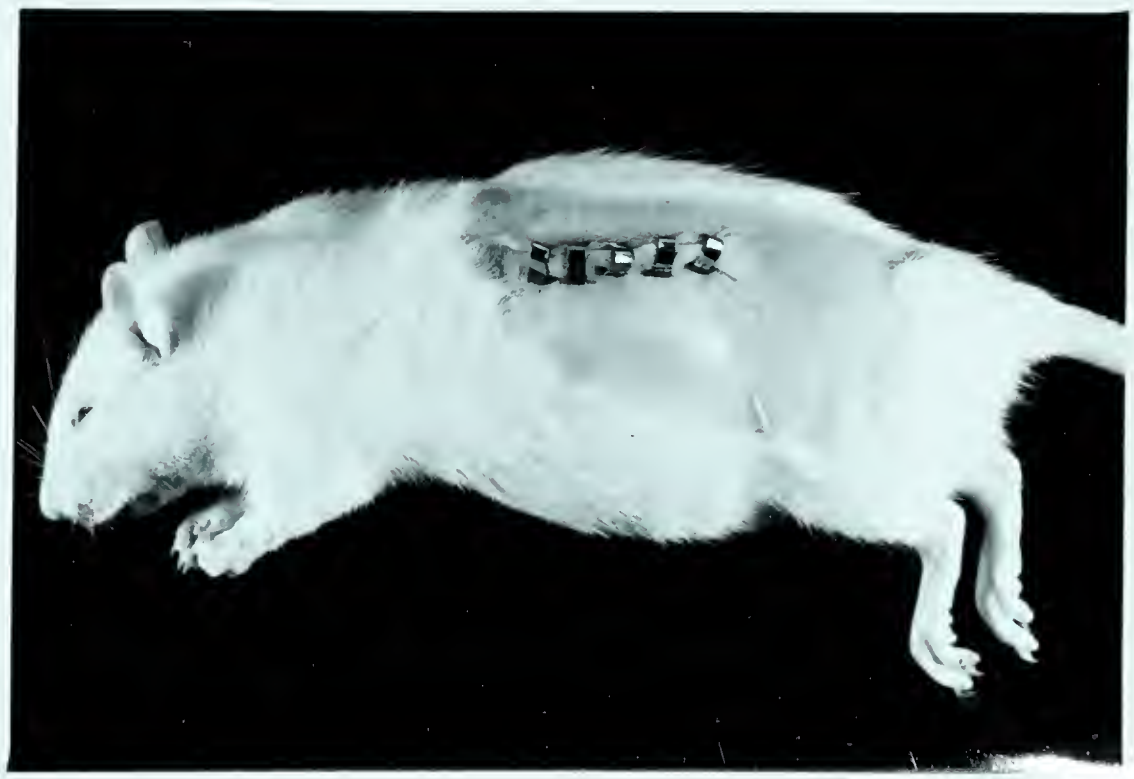

Figure 21: The tissue isolated tumor (26): Closure of the skin over the subcutaneous pocket.

0.15 ml. of the standard tumor suspension (page 1) was injected interstitially into the renal cortex through the paraffin envelope. The kidney was then returned to the subcutaneous pocket and the skin was reclosed with Michel clips. Four of the twenty-five rats were rejected because of the development of an abscess within and around the paraffin envelope. The remaining rats were left for a ten day interval to provide for the establishment of the tumor. At the end of this interval, twenty of the rats had obviously palpable tumors and were utilized in the following studies.

Fifteen of these rats were lightly anesthetized with Pentobarbital sodium (30 mg/kg) and prepared for the constant rate intravenous infusion technique of bloodflow determinations (page 21). In brief, PE90 polyethylene cannulas were placed in the left femoral artery and vein and connected respectively to withdrawal and infusion syringes of a modified Harvard 600-930 syringe pump (page 21). The infusion of approximately 10 micro-curies of 4-Iodoantipyrine and simultaneous withdrawal of an arterial sample were accomplished over a seventy second interval. At the termination of this interval the animal was sacrificed and the circulation was terminated by the infusion of 2.0 ml. of a saturated solution of Potassium

chloride through a side-arm on the intravenous cannula. The right kidney and the enclosed left renal tumor were rapidly excised, weighed, and the concentration of indicator determined by counting in a well type scintillation counter for five minutes. Indicator concentrations were expressed as counts per minute per gram and bloodflow to these tissues was determined as previously (page 20) by the following equation:

$$\text{Flow (ml/min/gm)} = \frac{\text{Tissue concentration}}{(\text{art. conc.} - \tfrac{1}{2}\ \text{tissue conc.})T}$$

(7, p. 20)

Bloodflows were tabulated in Table VII and Figure 22.

The second group of five rats were similarly prepared, but in addition a three inch long PE90 polyethylene cannula was placed in the left renal vein, (the vein draining all of the blood from the tissue isolated Walker 256 tumor) through an abdominal incision to provide for diversion of the blood draining the tumor. This provided for a direct method to determine tumor bloodflow for comparison with the flow determination by the indicator dilution method, and therefore provided a measure of the overall accuracy of the indicator dilution method. The renal vein cannula was led to a weighed glass tube positioned below the animal for gravity drainage. Blood draining from the

renal vein cannula prior to the indicator dilution bloodflow determinations was approximately replaced through the femoral vein cannula with blood from a donor animal to minimize the effects of bloodloss on the experimental animal. The systemic arterial pressure of the rat was not monitored during these determinations as we were only interested in comparing the indirect bloodflow determinations using Iodoantipyrine with those of the direct method provided by measuring the venous drainage from the tumor. At time zero in the infusion of indicator through the femoral vein cannula, a weighed collecting vessel was placed under the renal vein cannula, and at the end of the seventy second interval of indicator infusion, this vessel was reweighed to determine the volume of venous effluent.

The performance of indicator dilution bloodflow determinations was identical with that described above. The indirect bloodflow calculations were as described above. The results of the two methods are summarized in Table VIII.

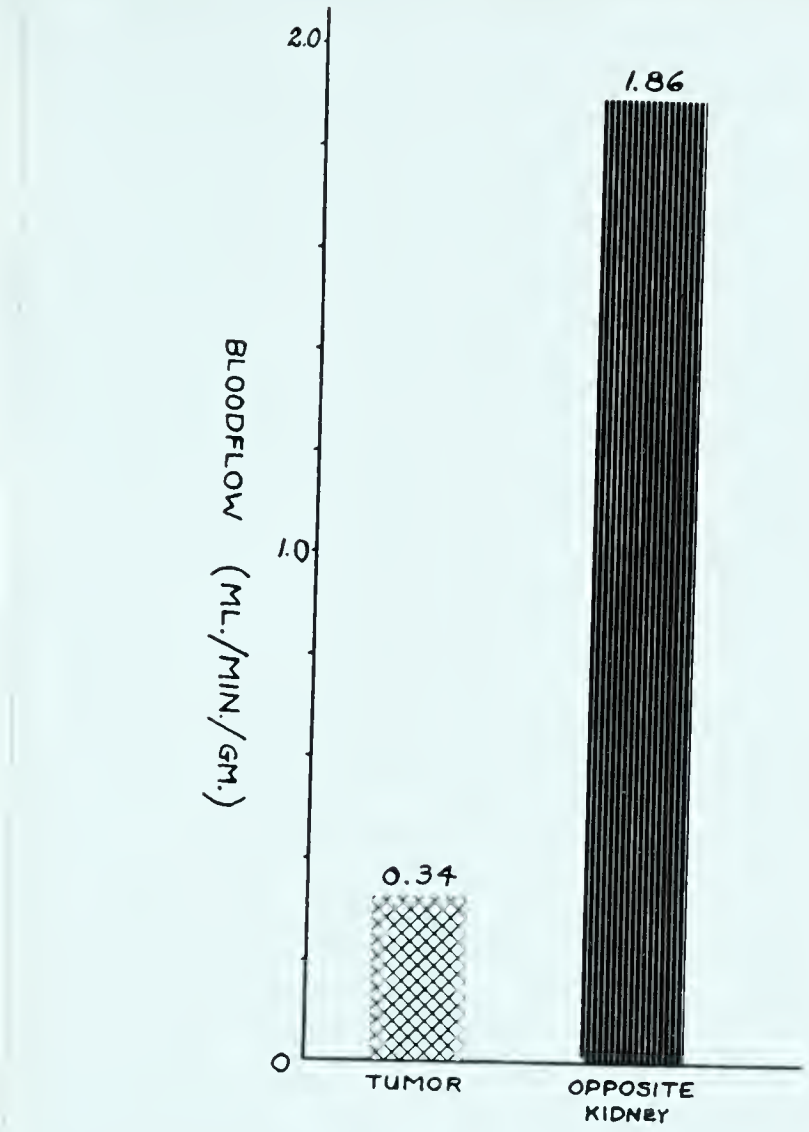


Figure 22: Bloodflow to the renal implant of the Walker 256 carcinomal compared with normal renal bloodflow.

Results:

TABLE VII

COMPARISON OF BLOODFLOW TO THE RENAL IMPLANT WITH THAT TO THE NORMAL KIDNEY AND TO THE INTRAMUSCULAR TUMOR IMPLANT

| TISSUE | GROUP SIZE | MEAN WEIGHT | MEAN BLOODFLOW (ml/min/gm) | STANDARD DEVIATION | RANGE |
|---|---|---|---|---|---|
| Renal tumor | 15 | 6.90 ± 0.27 | 0.34 | ± 0.017 | 0.22-0.41 |
| Kidney | 15 | 1.45 ± 0.25 | 1.86 | ± 0.048 | 1.50-2.31 |
| *I.M. tumor | 56 | | 0.27 | ± 0.009 | 0.15-0.41 |

*Results from page 25

TABLE VIII

BLOODFLOW TO THE TISSUE-ISOLATED RENAL TUMOR IMPLANT: DIRECT (VENOUS EFFLUENT) METHOD vs. INDIRECT (INDICATOR DILUTION) METHOD

| TISSUE | GROUP SIZE | TUMOR WEIGHT (gm.) | TUMOR BLOODFLOW (milliliters/min) | | |
|---|---|---|---|---|---|
| | | | Indicator-Dilution (ml/min/gm) | Total** | Venous Effluent |
| Renal tumor | 5 | 7.34 ± 0.51 | 0.33 ± 0.002 | 2.43 ± 0.32 | 2.48 ± 0.35 |

**Tumor weight x ml/min/gm.

Discussion:

The tissue-isolated tumor (26) described by Gullino and Grantham and utilized in this study, was investigated by these authors and compared with subcutaneous implants of the same tumors. These authors found the same correlation between wet weight, dry weight, and total nitrogen as well as the same level of anaerobic glycolysis. The host-tumor relationship was also apparently unaffected by growth of the tumor in this envelope, as measured by body weight change, food and water intake, and depression of liver catalase. It would appear that the tumor grown in this way remains indistinguishable from the same tumor grown in other sites.

In the foregoing experiment, the bloodflow to the tumor as a renal implant was compared with that to the intramuscular implant determined previously (page 25).

The tumor as a renal implant receives an average bloodflow of 0.34 $\pm$ 0.017 milliliters per minute per gram of tumor, as compared with the bloodflow to the tumor in an intramuscular site of 0.27 $\pm$ 0.009 ml./min./gm. The slight increase in bloodflow seen in the renal implant can probably be attributed to the presence of surviving areas of renal parenchyma which have a much higher bloodflow than the tumor, and which are always present in this

preparation as shown by Gullino and Grantham (27). The bloodflow to the tumor is very similar in these two sites of implantation.

The bloodflow to the opposite normal kidney was found to be 1.86 ± 0.048 ml/min/gm. This is in agreement with the generally accepted levels of renal bloodflow in the albino rat of 240 ml/hr (18, 60, 71). The bloodflow to the normal kidney is therefore approximately six times that to the tumor implanted in the kidney when expressed as ml. flow/minute/gram of tissue.

The evaluation of the accuracy of this indirect method for the determination of tissue bloodflows has been presented elsewhere (pp. 26-38 ). The use of the tissue isolated tumors provided a means for measuring the level of tumor bloodflow directly by measuring the venous outflow from the tumor preparation (Table VIII). The simultaneous measurement of venous outflow and tissue flow by the indicator dilution method as described above (pp. 41-42) has shown that there is very little overall error in the indirect method, as the indirect flow (2.43 ± 0.32 ml/min) was found to agree closely with the measured venous outflow (2.48 ± 0.35 ml/min).

The total tissue bloodflow to the tumor or kidney may be calculated by multiplying the flow per gram by the total

weight of the organ (Table VII). It is of interest that the total flow to the tissue isolated tumor (2.35 ± 0.22 ml./min.) is significantly less than the bloodflow to the opposite kidney (2.68 ± 0.50 ml/min.), although the tumor is approximately five times the weight of the normal kidney.

B. Bloodflow To the Lymphnode Metastases

The Walker 256 tumor, when implanted in the hind limb musculature, metastasizes to the regional lymph nodes (64, 68), the para-aortic nodes. The bloodflow to these metastases can be easily estimated by employing the constant rate intravenous infusion method using Iodoantipyrine (p. 21 ).

Method:

Rats bearing a fifteen day old Walker 256 carcinoma were found to have large para-aortic lymphnode metastases. Twenty such rats were prepared for the Iodoantipyrine method of tissue blood flow determinations (page 21 ) and the standard technique was used. At the termination of Iodoantipyrine infusion, the nodal metastases were removed and their tissue bloodflows were determined in the usual manner. The results are summarized in Table IX.

Results:

TABLE IX

THE TISSUE BLOODFLOW TO LYMPHNODE METASTASES OF THE WALKER 256 CARCINOMA

| TISSUE | GROUP SIZE | BLOODFLOW (ml/min/gm) | RANGE |
|---|---|---|---|
| lymphnode tumor | 20 | 0.29 ± 0.011 | 0.27-0.38 |
| renal tumor | 15 | 0.34 ± 0.017 | 0.22-0.41 |
| muscle tumor | 56 | 0.27 ± 0.009 | 0.15-0.41 |

Conclusions:

The bloodflow to the Walker 256 carcinoma is remarkably uniform when implanted in three markedly different tissues, namely, hind limb musculature, kidney, and lymphnode metastases as is summarized in Table IX (page 50). The magnitude of tumor bloodflow is not determined by the tissue in which the tumor is implanted, but is an inherent property of the tumor.

# CHAPTER IV

## THE REGULATION OF TUMOR BLOODFLOW

The experiments discussed in the preceding chapters have shown that the Walker 256 tumor has a low value of tissue bloodflow which is characteristic of this tumor and is not modified by the site of implantation. Studies of tumor vasculature employing arteriography and vascular casting techniques have suggested that the tumor develops its blood vessels from those of the host tissue.

This low value of tumor bloodflow when compared to host tissues is important when considering antitumor chemotherapy where the agent is delivered to the tumor in the bloodstream, as tumor exposure to the agent is relatively small.

It is therefore important to compare the reactions of tumor vessels as measured by the tissue bloodflow to certain procedures and to the administration of certain drugs with those of normal tissues, both to determine whether tumor vessels are capable of responding to such stimuli, and to determine whether tumor vessels respond to these stimuli in a manner which is different from those of the normal tissues. Differences in the response of tumor vessels to such stimuli may be exploitable to enhance the

effects of antitumor chemotherapy.

In the following study, the alterations in tumor tissue bloodflow as a hind limb implant produced by lumbar sympathectomy and by the systemic administration of a variety of vaso-active chemicals was compared with alterations produced in the tissue bloodflows to normal muscle and skin produced by these same procedures.

A. The Effect of Lumbar Sympathectomy on Bloodflow to the Tumor and to the normal tissues of the hind limb

The vessels of the hind limb, especially the small muscular arteries and arterioles are supplied with sympathetic fibers which are derived almost completely from the lumbar sympathetic ganglia (15, 35). In the normal physiological state, these fibers exert a tonic vasoconstrictor action on the hind limb vessels (3, 24). Extirpation of these lumbar sympathetic ganglia produces vasodilatation in the hind limb vessels with increased regional bloodflow consequent to the removal of this tonic vasoconstrictor action (3, 35). This vasodilatation and increased regional bloodflow is more marked in the vessels of the skin than in those of the muscle (3, 15, 35).

In the following study, the tumor is implanted in the hind limb musculature (page 2 ) and presumably developes

its vessels from those of muscle. The effects of lumbar sympathectomy on tissue bloodflow to the tumor were compared with those to muscle and skin.

Methods:

A group of male Sprague-Dawley rats were lightly anesthetized with diethyl ether and prepared by shaving the abdominal skin and painting with tincture of Iodine. A long midline incision was made and the viscera were reflected to the right side. The posterior peritoneum was incised along the left border of the aorta, and dissection was carried into the central groove between the two sacrospinalis muscle groups until the two lumbar sympathetic chains were identified. These chains were then excised from the level of the diaphragm to the pelvis. The peritoneum was closed with a continuous suture of fine gut and the abdominal incision was closed.

On the fourth post-operative day, the rats were examined and those with obviously pink warm feet were chosen for tumor implantation. This difference in skin color of the feet when compared with the normal rat is very marked. The Walker 256 carcinoma was implanted into the hind limb musculature of the sympathectomized rats as described on page two .

Fifteen sympathectomized rats bearing a six day old

tumor were prepared for the infusion method of determining tissue bloodflow, as detailed previously (page 25). Tissue bloodflows to the tumor, hind limb muscle and skin were calculated as previously described and are summarized in Table X.

Results:

TABLE X

THE EFFECTS OF LUMBAR SYMPATHECTOMY ON BLOODFLOW TO TUMOR, MUSCLE AND SKIN IN THE RAT HIND LIMB

| TISSUE | TISSUE BLOODFLOW (ml/min/gm) Sympathectomy | *Controls |
|---|---|---|
| Tumor | 0.33 ± 0.029 | 0.27 ± 0.009 |
| Muscle | 0.36 ± 0.028 | 0.38 ± 0.008 |
| Skin | 0.31 ± 0.008 | 0.23 ± 0.008 |

*Page 25

Discussion:

Extirpation of the lumbar sympathetic ganglia produces an immediate flushing and warming of the skin with an eightfold increase in skin bloodflow as measured by venous occlusion plethysmography (3). This marked increase in skin bloodflow is not seen in muscle where the flow is only doubled (3, 35). This local vasodilatation rapidly subsides (in the human) to reach a level of only

double the control skin flows within two or three months (3).

In man and in the rat, lumbar sympathectomy produces only a transient fall in the arterial blood pressure which returns to normal values in one to several days (35).

In the experimental preparation used in this study, lumbar sympathectomy produced no change in muscle bloodflow when compared with the normal animal. Tumor bloodflow was increased somewhat to 0.33 $\pm$ 0.029 ml./min./gm. from the control flow of 0.27 $\pm$ 0.009 ml./min./gm. This increase in tumor bloodflow is less than the increase in skin flows which increased from 0.23 $\pm$ 0.008 ml./min./gm. to a level of 0.31 $\pm$ 0.008 ml./min./gm. The vessels of the tumor receive sympathetic vasoconstrictor fibers which apparently exert a tonic effect as in normal tissues. The increase in tumor bloodflow produced by lumbar sympathectomy is unexpected in view of the lack of increase in muscle bloodflow.

The demonstration of the change in tumor bloodflow produced in this experiment by lumbar sympathectomy implies that the tumor vessels receive sympathetic innervation and therefore presumably the tumor bloodflow responds to the central mechanisms regulating peripheral bloodflow through sympathetic pathways. The vessels of the tumor are not abnormal in this respect.

B. The Effect of a Variety of vaso-active drugs on tissue Bloodflow to muscle, skin and to the Walker 256 Tumor in the rat hind limb.

The chief mechanisms for the regulation of bloodflow through the peripheral capillary beds involve alterations in the caliber of the small vessels at the termination of the arterial tree (77). The smooth muscle of the terminal arterioles, meta-arterioles and pre-capillary sphincters receive sympathetic innervation at least as distal as the meta-arterioles (77, 15, 24), and are also capable of reacting directly to certain chemical agents such as epinephrine and norepinephrine (15, 17).

As seen in chapter one, the vascular tree of this transplanted tumor develops in part from the vessels of the host tissue, and a second portion which follows cellular growth. It is important to compare the reactions of the tumor vascular bed to the actions of certain chemicals with those of the normal tissues, insofar as changes in capillary bed bloodflow are concerned.

Method:

Tissue bloodflows to the muscle, skin and to the implanted tumor in the rat hind limb were measured using the constant rate intravenous infusion method employing 4-Iodoantipyrine as previously described (page 21).

In brief, the left femoral artery and vein of the experimental rat were cannulated with PE90 polyethylene cannulas which were connected to withdrawal and infusion syringes of a Harvard model 600-930 syringe pump. Approximately ten micro-curies of 4-Iodoantipyrine was infused at a constant rate into the femoral vein cannula for a period of seventy seconds. At the termination of this period, the right (tumor-bearing) hind limb was separated from the circulation with heavy shears and samples were taken from the lateral skin, the soleus muscle and the tumor implant. These samples were weighed to 0.1 mg. and indicator concentrations were determined by counting for five minutes in a well type scintillation counter. Indicator concentrations were expressed as counts per minute per gram, and tissue bloodflows were calculated by means of equation seven (page 20). Certain drugs (epinephrine and levarterenol) were administered as a continuous intravenous infusion during the period of bloodflow determinations through a side-arm on the femoral vein cannula. The experimental groups of tumor-bearing rats received intravenous doses of a variety of drugs prior to the bloodflow determinations. The drugs employed, dosages and method of administration are summarized below in Table XI. Epinephrine was administered in two different ways because of the unexpected

finding of muscle vaso-constriction with a decrease in muscle bloodflow. This drug acts as a transient vaso-constrictor (2, 4, 17) in muscle, later acting as a vaso-dilator (2, 4, 17). In the first group, (a), the drug was administered as a continuous infusion into the femoral vein cannula beginning at time zero of the bloodflow determinations. In the second group, (b), the infusion of epinephrine was begun five minutes before the determination of bloodflow.

TABLE XI

DRUG DOSAGE AND METHOD OF INTRAVENOUS ADMINISTRATION

| AGENT | GROUP SIZE | DOSAGE (mg/kg) | METHOD OF ADMINISTRATION |
|---|---|---|---|
| Levoarterenol | 9 | 0.05 | Continuous infusion at T=0 |
| Priscoline (Tolazoline) | 10 | 0.75 | I.V. at T=0. |
| *Epinephrine (a) | 10 | 0.05 | Continuous infusion at T=0 |
| (b) | 10 | 0.10 | Continuous infusion at T= -5 |
| Dibenzylene (Phenoxybenzamine) | 10 | 2.00 | I.V. at T= -5 |
| Chlorpromazine | 11 | 0.75 | I.V. at T= -5 |

*See text above.

The arterial pressure of the experimental animals was not monitored during the period of bloodflow determinations as the desired information was a comparison of

tissue bloodflows to tumor, muscle and skin following the administration of these drugs. No comparison is intended between the actions of the different drugs. For purposes of the discussion, the effect of these dosages and methods of drugs on blood pressure measured in a separate group of rats. The mean carotid blood pressure was measured during the period which would be occupied with the bloodflow determinations and is summarized in Table XII.

TABLE XII

THE EFFECT OF THESE DRUGS ON THE MEAN CAROTID PRESSURE IN THE RAT

| AGENT | GROUP SIZE | MEAN CAROTID PRESSURE (mm. Hg.) |
|---|---|---|
| Control | 2 | 91 |
| Levoarterenol | 2 | 144 |
| Priscoline | 2 | 94 |
| *Epinephrine (a) | 2 | 136 |
| (b) | 2 | 130 |
| Dibenzylene | 2 | 70 |
| Chlorpromazine | 2 | 66 |

Drug dosages and methods of administration given in Table XI.

*See text (pp. 58-59).

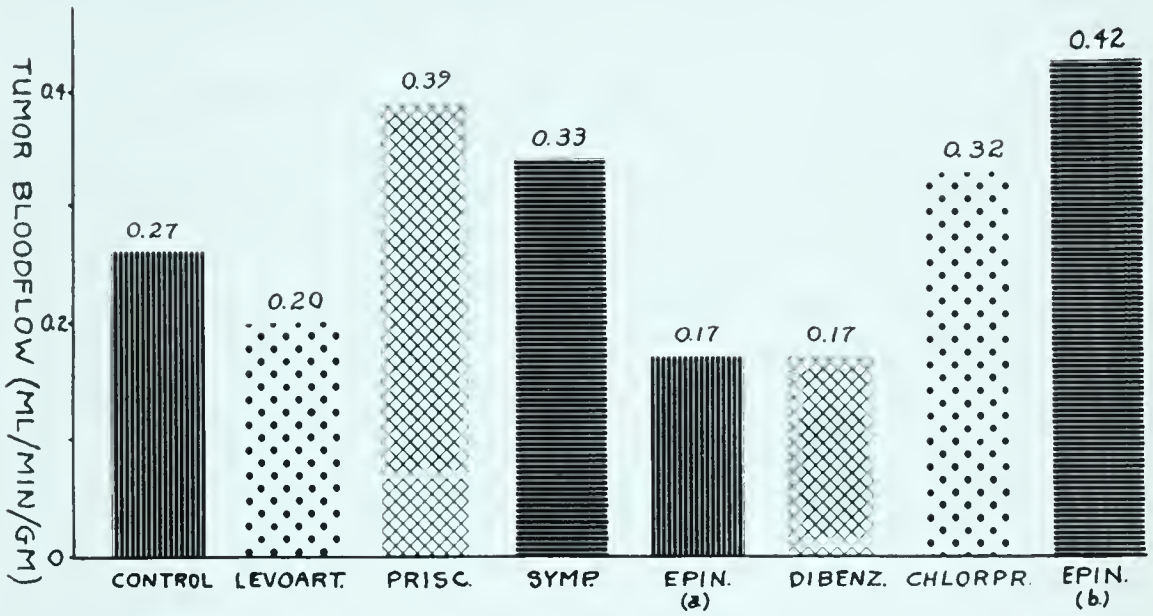


Figure 23: The effect of a variety of stimuli on the capillary-bed bloodflow to the Walker 256 carcinoma implanted in the hind limb musculature.

Control--untreated animals
Levoart.--Levoarterenol
Prisc.--Priscoline (Tolazoline)
Symp.--Lumbar sympathectomy
Epin. (a)--Epinephrine, immediate*
Dibenz.--Dibenzylene (Phenoxybenzamine)
Chlorpr.--Chlorpromazine
Epin. (b)--Epinephrine, later*

*see text

Results:

The resultant tissue bloodflow were calculated as previously described and are tabulated below (Tables XIII-XV). The control bloodflows included in the tables are those flows determined in Chapter I (page 25), using the same method for determination. The values listed under the heading "percentage change" are the percentage of change in bloodflow to tumor, muscle and skin produced by these drugs as compared to the control bloodflows to these same tissues. This column has been added for ease in comparing the control flows with those resulting from the administration of the drugs.

TABLE XIII

THE EFFECT OF A VARIETY OF DRUGS ON THE CAPILLARY-BED BLOODFLOW TO THE WALKER 256 CARCINOMA IN THE RAT HIND LIMB

| GROUP | GROUP SIZE | TUMOR BLOODFLOW (ml.min./gm.) | % Change* |
|---|---|---|---|
| **Control | 56 | 0.27 ± 0.009 | --- |
| Levoarterenol | 9 | 0.20 ± 0.011 | -26.0 |
| Priscoline | 10 | 0.39 ± 0.010 | +44.4 |
| ***Epinephrine (a) | 10 | 0.17 ± 0.007 | -37.0 |
| (b) | 10 | 0.42 ± 0.019 | +55.5 |
| Dibenzylene | 10 | 0.17 ± 0.012 | -37.0 |
| Chlorpromazine | 11 | 0.32 ± 0.009 | +19.5 |

*See text above.

**Controls from page .

***See text (page 59).

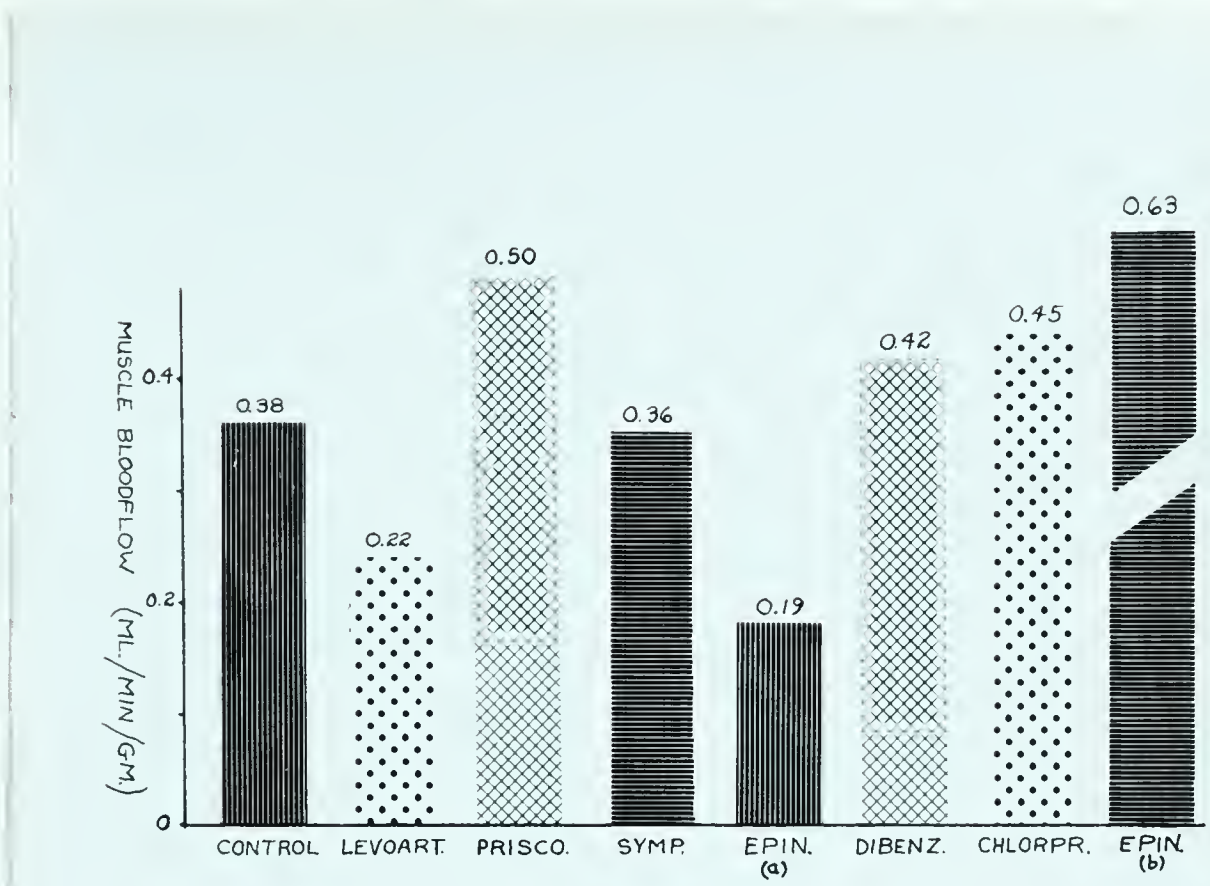


Figure 24: The effect of a variety of stimuli on the capillary-bed bloodflow to skeletal muscle in the rat hind limb.

Control--untreated animals
Levoart.--Levoarterenol
Prisc.--Priscoline (Tolazoline)
Symp.--Lumbar sympathectomy
Epin. (a)--Epinephrine, immediate*
Dibenz.--Dibenzylene (Phenoxybenzamine)
Chlorpr.--Chlorpromazine
Epin. (b)--Epinephrine, later*

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*see text

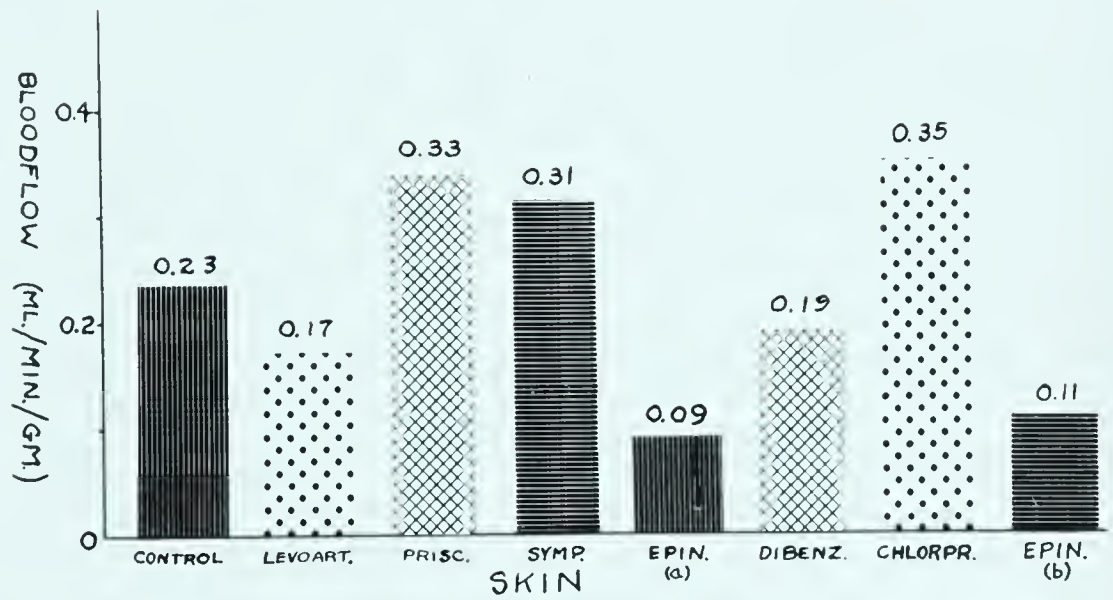


Figure 25: The effect of a variety of stimuli on the capillary-bed bloodflow to skin of the rat hind limb.

Control--untreated animals
Levoart.--Levoarterenol
Prisc.--Priscoline (Tolazoline)
Symp.--Lumbar sympathectomy
Epin. (a)--Epinephrine, immediate*
Dibenz.--Dibenzylene (Phenoxybenzamine)
Chlorpr.--Chlorpromazine
Epin. (b)--Epinephrine, later*

---

*see text

TABLE XIV

THE EFFECT OF A VARIETY OF DRUGS ON THE CAPILLARY-BED BLOODFLOW TO MUSCLE IN THE RAT HIND LIMB

| GROUP | GROUP SIZE | MUSCLE BLOODFLOW (ml.min./gm.) | % Change* |
|---|---|---|---|
| **Control | 56 | 0.38 ± 0.008 | --- |
| Levoarterenol | 9 | 0.22 ± 0.009 | -42.1 |
| Priscoline | 10 | 0.50 ± 0.013 | +31.6 |
| ***Epinephrine (a) | 10 | 0.19 ± 0.010 | -50.0 |
| (b) | 10 | 0.63 ± 0.015 | +65.8 |
| Dibenzylene | 10 | 0.42 ± 0.019 | +10.5 |
| Chlorpromazine | 11 | 0.45 ± 0.013 | +18.9 |

TABLE XV

THE EFFECT OF A VARIETY OF DRUGS ON THE CAPILLARY-BED BLOODFLOW TO SKIN IN THE RAT HIND LIMB

| GROUP | GROUP SIZE | SKIN BLOODFLOW (ml./min./gm.) | % Change* |
|---|---|---|---|
| **Control | 56 | 0.23 ± 0.008 | --- |
| Levoarterenol | 9 | 0.17 ± 0.017 | -26.1 |
| Priscoline | 10 | 0.33 ± 0.009 | +43.5 |
| Epinephrine (a) | 10 | 0.09 ± 0.009 | -60.9 |
| (b) | 10 | 0.11 ± 0.008 | -52.2 |
| Dibenzylene | 10 | 0.19 ± 0.019 | -17.4 |
| Chlorpromazine | 11 | 0.35 ± 0.013 | +52.2 |

*
** as for Table XIII
***

Discussion:

The administration of Levoarterenol (l-norepinephrine) in a dose of 0.05 mg./kg. by continuous intravenous infusion produced a change in the capillary bed (tissue) bloodflow to the Walker 256 carcinoma from a control level of 0.27 ± 0.009 ml. per minute per gram of tissue to a level of 0.20 ± 0.011 ml./min./gm., an alteration of -26.0 percent. This drug produced a change in the tissue bloodflows to muscle and to skin (Tables XIV, XV), compared to control levels, of -42.1% and -26.1% respectively. Levoarterenol is the predominant substance present at sympathetic endings, and acts directly on the effector cells, producing vasoconstriction of all vessels (2, 4).

Priscoline (Tolazoline), when administered in an intravenous doses of 0.75 mg./kg., produced an increase in tumor bloodflow from a control level of 0.27 ± 0.009 to 0.39 ± 0.010 ml./min./gm., a change of +44.4%. This same drug produced increases in the tissue bloodflows to muscle and to skin of +31.6% and +43.5%. This drug produces the rapid onset of a peripheral adrenolytic action and a direct local vasodilatation (4, 20). The action was to increase the capillary-bed bloodflows to tumor, muscle and to skin of the rat hind limb.

Epinephrine is a potent vaso-active drug acting directly on the effector cells to produce an increase in cardiac output, an increase in arterial pressure, and to constrict most peripheral vessels (2, 4, 17, 20). This drug acts as a transient vasoconstrictor in skeletal muscle blood vessels and later always as a vasodilator (2, 4, 17). In the first series of experiments using Epinephrine (series a, page 58), the drug in a dose of 0.05 mg./kg. was administered to the experimental animal as a continuous infusion into a side-arm of the femoral vein cannula, beginning at time zero of the bloodflow determinations. Epinephrine administered in this manner produced a marked elevation of carotid pressure (Table XII) to 136 mm. Hg., from a control level of 91 mm. Hg., but nevertheless produced a marked decrease in bloodflows to the tissues of the hind limb and tumor. The changes in bloodflows produced by the drug in this instance were -37.0%, -50.0%, and -60.9% to tumor, to muscle and to skin respectively.

Other investigators (above) have shown that this drug (epinephrine) produces a transient vasoconstriction in skeletal muscle blood vessels, followed by pronounced vasodilatation. The experiments using this drug were repeated in a second group of ten tumor-bearing rats utilizing a dose of 0.10 mg./kg. administered as a continuous

infusion into the side-arm of the femoral vein cannula, beginning five minutes before the determination of tissue bloodflows (T = -5).

The administration of Epinephrine in this manner produced a marked increase in bloodflow to skeletal muscle and to the tumor with a marked decrease in skin bloodflow, confirming the vasodilating nature of the action of this drug on skeletal muscle blood vessels. The changes in bloodflow to tumor, to muscle, and to skin were +55.5%, +65.8% and -52.2%, expressing the change as the percentage change of control bloodflow. It is important to note that this drug produced a change in tumor bloodflow which paralleled that of skeletal muscle but was in the opposite direction to that observed in the skin. This supports the hypothesis that the vessels of the tumor are developed from those of the host tissue and retain the functional integrity and properties of host tissue vessels.

Dibenzylene (Phenoxybenzamine) administered in a dose of 2.0 mg/kg as a single intravenous injection five minutes before the determination of tissue bloodflows (T = -5) produced a marked decrease in tumor bloodflow from a control level (Table XIII) of 0.27 $\pm$ 0.009 to 0.17 $\pm$ 0.012 ml./min./gm., a change of -37.0%. Bloodflow to muscle and to skin were altered +10.5% and -17.4%, compared

to control levels. This drug is an adrenergic blocking agent, blocking all excitatory responses to sympathetic stimulation (4, 20) and would be expected to produce peripheral vasodilatation and consequent increased peripheral bloodflows. Dibenzylene produced a marked fall in the carotid arterial pressure of a different group of anesthetized rats, to a mean of 70 mm. Hg. from a mean of 91 mm. Hg. in the untreated animal. This fall in systemic arterial pressure probably explains the decreased peripheral bloodflows. The discrepancy between the moderate reductions in bloodflows to tumor and skin and the slight increase in muscle bloodflow is statistically significant at the 5% level and is not explained.

Chlorpromazine, when administered as a single intravenous dose of 0.75 mg./kg. five minutes before in determination of tissue bloodflows produced a moderate increase in the bloodflow to tumor, muscle and skin of +19.5%, +18.9%, and +52.2% respectively. It is of interest that this drug produced a much greater increase in the bloodflow to skin than to muscle or to the tumor.

Chlorpromazine produces a widespread decrease in sympathetic activity and acts as a direct peripheral vasodilator (4, 20). The greater sympathetic tonic vasoconstrictor action on the blood vessels of skin than on those

of muscle (11, 15, 77) probably explains the greater increase in skin bloodflows than in muscle which is exhibited in this experiment.

In summary, changes in bloodflow to the Walker 256 Carcinoma implanted in an intramuscular site, to skeletal muscle and to skin in the rat hind limb were compared following the administration of levoarterenol, Priscoline, Epinephrine, Dibenzylene and Chlorpromazine. Epinephrine in the first minute of continuous administration, Levoarterenol, and Dibenzylene were found to produce a reduction in the tissue bloodflow to the tumor (and to muscle and skin). The reduction seen with Dibenzylene is probably attributable to a decrease in systemic arterial pressure produced by this agent.

Epinephrine after five minutes of continuous administration, Priscoline (Tolazoline) and Chlorpromazine were found to produce an increase in tumor bloodflow. In all cases, the increase in tumor bloodflow was roughly parallel to that seen in skeletal muscle. Epinephrine after five minutes of continuous administration was found to produce a marked increase in tissue bloodflow to the tumor and to skeletal muscle while producing a marked decrease in skin bloodflow.

The vessels of the intra-muscular tumor are

functionally the same as skeletal muscle vessels, confirming the findings of arteriography and vascular casting techniques (pages 3-9) that the tumor develops its vessels from those of the host tissue.

In studies of the mouse Sarcoma 180 and of spontaneous mouse mammary carcinomas, Nataze (50) found that epinephrine increased tumor bloodflow by increasing vessel diameters and the linear rate of flow through these vessels. Acetylcholine and Histamine had an opposite effect. Nataze utilized skin calorimetry and direct observation of tumor vessels in tumor fragments transplanted into a transparent chamber. He interpreted these results as showing that the tumor vessels respond in an opposite manner compared with the vessels of "normal" tissues. The measurement of skin temperature overlying subcutaneous neoplasms is an unreliable measure of tumor bloodflow. Similarly, the transparent chamber provides an abnormal environment for the tissues, and changes seen in the vessels in this site may not be representative of those of the tumor as a whole. The diameter and linear flow rates of small vessels are profoundly influenced by local conditions and metabolites (77). It is of interest that Nataze did not observe vasoconstriction in the "normal" vessels when Epinephrine was administered by iontophoresis.

The findings of Bierman et. al. (6) must also be rejected on the basis that variations in skin temperature in the skin overlying superficial neoplasms are not directly related, if related at all, to tumor bloodflow.

Gullino and Grantham (28) investigated the response of a variety of animal tumors, including the Walker 256 tumor, as tissue-isolated renal and ovarian implants to epinephrine, acetyl-B-methylcholine, cold and celiac ganglionectomy by directly measuring the venous out-flow from the tumor. They demonstrated that acetyl-beta-methylcholine produced an increase in bloodflow to the tumor and to the opposite (normal) host organ, while the other stimuli produced a reduction. The vessels of the tumor responded in the same manner as the vessels of the host organ, a conclusion which is in agreement with the findings of the above experiment. It is of interest that these investigators found that Epinephrine produced a vasoconstriction and decrease in tumor bloodflow with the tumors implanted in the ovary and kidney, in view of the increased flow seen to the intra-muscular tumor produced by Epinephrine here.

The vessels of the tumor therefore respond to a variety of stimuli in the same manner as do those of the host tissue. This is not unreasonable as the vessels are the same.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

The clinical usage of methods for the treatment of malignant neoplasms where anti-tumor chemotherapeutic agents are introduced into the arterial blood supply of the tumor (13, 45) has provided few "cures". These disappointing results have led to a re-examination of certain long cherished beliefs regarding the magnitude and the regulation of bloodflow to malignant neoplasms.

It has been generally accepted that the blood supply to malignant tumors is greater than that to most normal tissues, a belief supported by a number of earlier investigations where the magnitude of tumor bloodflow was either interpolated from a knowledge of tumor vascular architecture provided by arteriography or by vascular casting techniques (5, 9, 14, 29, 34, 62), or by the use of a variety of indirect methods for estimating tumor flow. Such methods as the measurement of the skin temperatures overlying superficial neoplasms (6, 50), the determination of tumor oxygen tensions or A-V oxygen differentials (7, 69, 70), or the observed distribution of certain dyes within the tumor (21, 34, 51) do not provide a direct measurement of the amount of blood perfusing the capillary bed of

the tumor.

Recent studies employing more accurate methods have shown that a number of transplantable animal tumors have less blood supply than do most normal tissues (27, 28, 61).

The regulation of tumor bloodflow has been shown to be abnormal, that is, the response of the tumor vessels and of tumor bloodflow to a variety of drugs was demonstrated to be different than those of adjacent normal tissues (6, 50), thus presenting the concept that the tumor exposure to chemotherapeutic agents may be increased by pre-treatment with vasoactive drugs. This concept has also been shown to be unfounded (28), when more accurate methods of measuring tumor bloodflow have been applied to this problem. The response of tumor bloodflow has been shown to be identical to that of the host tissue when challenged by a variety of stimuli (28).

In this study, the blood supply of a rapidly growing, transplantable rat tumor, the Walker 256 carcinoma, implanted into the right hind limb musculature or into the left kidney of Sprague-Dawley rats, was investigated by a number of methods.

The architecture of the vascular supply of this tumor as a hind limb implant was studied through iliac arteriograms using metallic mercury as the contrast agent

and by producing vinyl plastic casts (47, 72) of the arterial and venous trees of the hind limb. The Walker tumor was found to be relatively avascular; to develop its vessels from the pre-existing skeleton of host tissue vessels; and to be supplied by small vessels which enter the tumor from the periphery. Radioautograms of cross-sections of the tumor-bearing limb following the intra-arterial infusion of radioactive ($Sc_{46}$) ceramic microspheres (43, 74) of 25 $\pm$ 5 microns diameter demonstrated that the tumor is not penetrated by vessels of greater than this diameter, as the microspheres were observed to be clustered around the periphery of the tumor. The small size and peripheral orientation of the supply vessels of this tumor implies that the central portions of the tumor are relatively avascular and therefore have relatively poor access to nutrients or anti-tumor drugs carried in the arterial blood stream. These findings may explain the disappointing results of regional anti-tumor chemotherapy (68), and also the occurrence of central necrosis in this fast growing neoplasm.

The capillary bed bloodflow to a region of the body may be estimated by means of indicator dilution techniques. An indicator is a substance which is introduced into the vascular system of an experimental animal in a known dose

and becomes diluted by the flow of blood past the point of injection. It has been shown (16, 32, 33, 37, 38, 39, 40, 41, 42, 44, 55, 76) that the determination of the concentration of indicator substance in the blood at a point downstream from the plane of injection over a given time interval enables one to determine the quantity of blood flowing past the point of injection over that interval, if the assumption of complete mixing may be made. If a freely diffusible indicator is mixed into the arterial bloodstream, the indicator substance will accumulate in the tissues of the body in proportion to the bloodflow through their respective capillary beds (12, 23, 27, 41, 42, 52). From a knowledge of the arterial concentrations and the tissue concentrations of indicator, the local tissue (capillary bed) bloodflow may be calculated.

In this study, capillary bed bloodflow to the normal tissues of the rat hind limb and to the hind limb tumor implant were estimated in fifty six rats using these methods employing a diffusible indicator, Radio-4-Iodoantipyrine $I_{131}$ (Abbott). The method of bloodflow determination was that developed by Aust and co-workers (53, 61) and is briefly summarized here;

The indicator was infused into the left femoral vein of the experimental rats at a constant rate, while arterial

concentrations were measured by simultaneous withdrawal of an arterial sample at a constant rate from the left femoral artery. The infusion of indicator and withdrawal of arterial sample took place over a seventy second interval at the termination of which the right (tumor-bearing) hind limb was rapidly amputated with heavy shears. Indicator concentrations were determined in the arterial sample and in samples of tumor, skeletal muscle and skin by counting in a well type scintillation counter and were expressed as counts per minute per gram of sample. Tissue blood flows were calculated from the following expression (53, 61):

$$Q = \frac{A}{(\overline{Ca} - \tfrac{1}{2}A)T}$$

where Q=flow in ml/min/gm.
A=tissue conc. in cpm/gm.
Ca=mean arterial conc. in cpm/gm.
T=time in minutes.

The derivation of this formula is discussed in the text. The major sources of error in this method involve assumptions regarding the time course of the arterial and venous concentrations, the rate and extent of diffusion of the indicator and the tissue extraction of the indicator. These were investigated in a separate series of experiments. This method proved to be free of errors of any great magnitude.

The bloodflow to the tumor (0.27 $\pm$ 0.009 ml./min./gm.)

was found to be considerably smaller than that to resting skeletal muscle (o.38 ± 0.008) and only slightly higher than that to skin (0.23 ± 0.008). The bloodflow to the tumor in other sites of implantation was determined using this same method of estimation. Thus, the bloodflow to the tumor as a renal implant (0.34 ± 0.017 ml. min./gm. and to metastases from the hind limb tumor to the para-aortic lymphnodes (0.29 ± 0.011) were determined and found to be similar to the flow to the tumor in the intra-muscular site of implantation. The tumor has relatively little bloodflow and the magnitude of tumor bloodflow is an inherent property of the tumor and is not significantly influenced by the site of implantation.

The tissue bloodflow to the opposite normal kidney was determined by the same method and was found to be approximately six times that to the renal tumor implant (1.86 ± 0.048 vs. 0.34 ± 0.017). This value for the normal renal bloodflow is in agreement with generally accepted measurements in the literature (18, 27, 60, 71) achieved by other methods.

The overall accuracy of this indicator dilution method for estimating tissue bloowflows was investigated by comparison of indicator-dilution bloodflow determinations with simultaneous measurement of the venous effluent

draining from specially prepared renal tumor implants. The renal tumor implants were provided with a paraffin envelope (26) enclosing the kidney and tumor and thereby producing a tumor supplied by a single artery and drained by a single vein, the renal artery and vein respectively. The indirect (indicator dilution) bloodflow determinations (2.43 $\pm$ 0.32 ml./min.) were in agreement with those determined by the direct measurement of the venous blood draining from the tumor (2.48 $\pm$ 0.35 ml./min.), showing that the indirect method contains no errors of large magnitude.

The earlier studies of Nataze (50) and of Bierman (6) using skin calorimetry and direct observation of vessels in fragments of tumor implanted in transparent chambers were interpreted as showing that tumor vessels and tumor bloodflow respond differently than adjacent normal tissues to the actions of a variety of vasoactive drugs. These experimental results are at odds with those of other work indicating that the vessels of the tumor are developed from those of the host tissue (supra). Gullino and Grantham (28), using more accurate methods of measuring tumor bloodflow were unable to confirm this finding of abnormal reactivity of tumor vessels when compared with those of the host tissues.

The changes in tissue bloodflow to the Walker 256

carcinoma as an intra-muscular implant, its host tissue (skeletal muscle) and skin in response to sympathectomy and to the systemic administration of several vasoactive drugs were compared with the previously determined control flow levels for these tissues. The drugs employed in this study were epinephrine, l-norepinephrine, tolazoline, phenoxybenzamine, and chlorpromazine. The effect of these drugs on the mean arterial pressure of the rat was determined in a separate pilot experiment.

Lumbar sympathectomy in fifteen rats produced a moderate increase in the bloodflow to the tumor (0.33 ml./min./gm. vs. a control flow of 0.27 ml./min./gm), relatively little change in muscle bloodflow (0.36 vs. 0.38), and a more marked increase in skin bloodflow (0.31 vs. 0.23). The greater increase in skin than in muscle bloodflow is in agreement with established concepts of the sympathetic innervation of these tissues (3, 15, 24, 35, 71).

The administration of l-norepinephrine, although raising the mean carotid pressure of a pilot group of rats from 91 to 144 mm. Hg., produced a marked decrease in tissue bloodflows in ten rats to the tumor, muscle and skin of -26.0%, -42.1% and -26.1% respectively, expressing the change in flow as a fraction of the control flows in

untreated animals.

Phenoxybenzamine (dibenzylene) produced a remarkable fall in carotid pressure of the pilot group from 91 to 70 mm. Hg., and perhaps as a consequence produced a decrease in tissue bloodflow to tumor, muscle and skin of -37.0%, +10.5%, and -17.4%.

Tolazoline (Priscoline) and Chlorpromazine both produced an increase in the tissue bloodflows to tumor, muscle and skin of +44.4%, +31.6%, +43.5%, and +19.5%, +18.9%, +52.2% respectively. Both drugs act as direct peripheral vasodilators and produced increased bloodflow to all tissues in the hind limb (4, 20).

Epinephrine is a known vasodilator of skeletal muscle capillary beds (4, 15, 17, 24), while producing vasoconstriction in most other vascular beds. The administration of epinephrine to a group of ten rats produced an initial decrease in tissue bloodflows to tumor, and to skeletal muscle of -37.0% and -50.0% respectively, expressing the flow change as a percentage fraction of the control flows. These determinations were repeated in a second group of ten rats after a period of five minutes of continuous infusion of this agent, and a marked increase in skeletal muscle and tumors flows of +65.8% and +55.5% were demonstrated. The bloodflowto skin was

markedly decreased in both groups, with values of -60.9% and -52.2% being recorded.

In all the series of experiments studying the changes in tissue bloodflows in response to these stimuli, the bloodflow responses of the tumor and its host tissue (skeletal muscle) were roughly parallel. The vessels of the tumor showed responses which were closely similar to those of skeletal muscle but which was different from those of the skin following lumbar sympathectomy and following the administration of epinephrine and chlorpromazine. These findings support the hypothesis that the tumor develops its vessels from those of the host tissue--the vessels are therefore the same

It is of interest to note that epinephrine produced an increased bloodflow to the intra-muscle tumor utilized in this study, while this agent produced decreased flows to a renal implant of this and other tumors in the study of Gullino and Grantham (28), again demonstrating that the vessels of the tumor are those of the host tissue.

In summary, the conclusions drawn from this study of the blood supply to the Walker 256 carcinoma are listed below:

1. This tumor is supplied by vessels of small caliber which enter the tumor mass from the periphery.

2. This tumor has relatively less blood supply than that to most normal tissues.

3. The vessels of this tumor are developed from the vessels of the host tissue and retain their functional identity and responses to a variety of vasoactive chemicals. The regulation of tumor bloodflow is probably identical with that of the host tissue.

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